the problem of presenting their results in popular form, in so far as that can be done without damage to scientific accuracy.

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ROBERT A. MILLIKAN

THE CITY

Human Behavior in the Urban Environment

By Robert E. Park, E. W. Burgess, *and* Roderick D. McKenzie

with a bibliography by Louis Wirth

The city as a social entity has thus far been studied chiefly by writers of fiction, and this book has been written in the belief that the time has come for a more searching and scientific treatment. An investigation of human behavior in the city environment and a study of urban life—its physical organization, its occupations, and its cultures—this volume treats the city as a product of human nature, rather than a bare artificial mechanism.

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There is a valuable and extensive bibliography by Louis Wirth. This is a volume in the "University of Chicago Studies in Urban Sociology."

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INTERRELATION OF RELATIVE DAY LENGTH AND TEMPERATURE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 343

BASIL E. GILBERT

(WITH FIVE FIGURES)

Introduction

The reactions of the plant to its environment have furnished material for much speculation and scientific investigation. Many interesting scientific facts have resulted from experimentation, during which plants have been subjected to varying combinations of environmental factors. In recent years the phenomenon of the reaction of the plant to varying day lengths has been of outstanding importance. Beyond the fact, however, that it is known that such a phenomenon exists, little is known of the mechanics of the reaction or its relation to the other factors of environment. It has seemed possible that one or other of these factors may bring about the same formative changes as those produced by "relative day length," or failing the discovery of any one definite causal agency, some knowledge may be gained concerning the interaction of one factor with another. The object of this investigation has been an attempt to determine the importance of temperature in determining the time of the initiation of flower primordia in *Xanthium pennsylvanicum*.

The importance of relative day length, or photoperiodism as it has been called, has been stressed greatly as the determining factor in causing flowering, by GARNER and ALLARD (8). Concerning the

interrelation of day length and temperature, they recognize the fact that "temperature is undoubtedly the most important environmental factor in relation to the action of the light period on plant growth," and they cite experiments with soy beans grown under short day conditions and differing temperatures in support of this assertion. The conclusion is drawn that a lower temperature delayed the time of flowering. They also draw attention to the effect of temperature on respiratory activity, and suggest that "in this way the relation between the duration of the light period and the prevailing temperature may be of decisive importance." WANSER (24) claims that day length independent of temperature will control the type of growth in winter wheat, and that by regulating this environmental factor, jointing and heading may be controlled independently of the season. EATON (5) has recently found that the time of flowering of Peking soy beans can be influenced to an extent comparable with the differences due to varying the day lengths if the plants are subjected to high, low, and uncontrolled nightly temperatures. The situation at the present time seems well summarized by ADAMS (1), who suggests that "much further experimentation will be necessary before any conclusions of a general nature can be drawn as regards the effect of light on the time of flowering." He also concludes that the reproductive activity, as well as the other activities of the plant, depend largely on photosynthesis, and the amount of photosynthesis is largely dependent upon the intensity of the light, its duration, and the temperature.

Closely allied with the experimentation by varying external factors have been efforts to determine the metabolic conditions produced within the plant. Several workers have thrown light on the situation accompanying flower development. KLEBS (13), working with *Sempervivum Funkii*, suggests that at low temperatures soluble sugars accumulate, and thus produce a set of conditions which he calls "ripeness to flower." He assumes an equilibrium between assimilation and dissimilation, and that light and temperature act contrary to each other in influencing this equilibrium. He gives us no data, however, in support of his theory, FISCHER (7) suggests the possibility of a high carbohydrate nitrogen ratio favoring flowering. He also fails to substantiate his theory with data. With the

work of KRAUS and KRAYBILL (16) on the tomato, a more complete conception of the relationship between carbohydrate and nitrogen compounds is given. They have drawn attention particularly to the probability of both carbohydrate and nitrogen becoming limiting under certain conditions, and have shown that, with the tomato at least, a limitation of either of these conditions inhibits flowering. GURJAR (9) has found that the same results hold for tomato, turnip, and radish; and WOO (26) finds that large amounts of carbohydrates are paralleled by low amounts of nitrogen in *Amaranthus*. NIGHTINGALE (17) also reports results similar to those of KRAUS and KRAYBILL, and draws attention to the possibility of the greater significance of insoluble nitrogen than total or nitrate nitrogen. HARVEY and MURNEEK (10) and other workers in the horticultural field have endeavored to follow up these results with investigations on the fruit bud differentiation in fruit trees. They conclude that while the carbohydrate-nitrogen ratio should not be given a causal relationship in the bringing about of a particular formative change in a plant organ, the ratio is important as an indication of possible limiting situations within the plant. HOOKER and BRADFORD (11) object to any significance being given the ratio, either as a causal agency or a relationship of fundamental importance. Their argument is summarized and the present situation commented upon by SUMMERS (23), who is of the opinion that "the C/N ratio represents a real causal relationship between the nutrient reserves of a tree and the intensity of fruit production." Another summary is given by KNIGHT (14), who draws attention to the possibility of the law of limiting factors operating within the plant, actuated by environmental changes and evidenced by fluctuations in carbohydrates and nitrogen.

There have been few direct examinations of the formative effects of different temperatures. WALSTER (25) summarized the literature up to 1920, and, working with barley, adds his own conclusions that high heat supply coupled with high nitrogen supply inhibits culm formation. JOHNSTON (12) observed that the flowers on the east side of the giant cactus mature and open before those on the other portions of the crown. He found that there was a higher average temperature in the tissues of the east side, and concluded that "this

higher average temperature brings the tissue of the east side nearer the optimum for growth and thus is responsible for the earlier maturing of the flowers of this side." Following a survey of the habitats in which *Zostera marina* is found, SETCHELL (20) was able to demonstrate that the important factor in bringing about formative changes is temperature and not photoperiodism. In the case of reproduction, he found the temperature limits quite definitely marked. Temperatures of 15°–20° C. accompanied the inception of this stage in the life of the plant.

Cultural conditions and chemical methods

A. CULTURAL CONDITIONS.—*Xanthium pennsylvanicum* was chosen as a plant particularly suited for this sort of investigation. It had been observed to give definite responses to temperature, and also is one of the "short-day plants" listed by GARNER and ALLARD. In addition to such advantages, this plant produces a terminal staminate inflorescence, and thus the beginning of the reproductive stage is easily observed. In preparing the seeds for all growth experiments, the burs were cut open and the seeds removed and soaked over night; then the brown testa was readily removable, leaving the ivory white seeds for planting. By this means germination of both upper and lower seeds was insured. The soil used was the greenhouse potting loam, which contained much organic matter, and in no case could the nitrogen supply be considered as limiting. The plants were grown in 12-inch unglazed pots and were watered with city water. The temperature of the greenhouses was controlled by automatic thermo-regulators. Temperature records were kept throughout the complete experimental period, Friez thermographs being used. The degree of fluctuation, as shown by the data in the tables, gave a minimum difference of 10° F. between the warm and cool greenhouses.

No attempt was made to control the humidity of the houses, and quite a wide range was observed, varying according to the amount of sunshine and the hour of the day. In general, however, the relative humidity, as measured by wet and dry bulb thermometers, was somewhat higher in the cool house. Thus evaporation was greater in the warm atmosphere. The average possible hours of sunlight

and the maximum and minimum daily temperatures for growth experiments carried on during the summer in the garden were secured from the Government Meteorological Station located on the campus of the University of Chicago.

B. Chemical methods.—Microchemical tests were made on the germinating seedlings. The methods used were those of Eckerson (6). The fresh tissues were sectioned and immediately tested for fats, reducing sugars, insoluble protein, and amino acids. The methods followed in the macrochemical analyses in general were those of Koch (15). It was found necessary, however, to introduce certain modifications for plant tissue.

1. *Sampling.*—The plants were sampled at 7:30 P.M. The roots were severed and discarded and the remainder of the plants ground through a Nixtamel mill. Moisture samples were weighed out in duplicate in tared moisture dishes, care being taken to secure as homogeneous a mixture of the pulp as possible. Whenever the size of the sample permitted it was divided into two portions, and each placed in a 250 cc. tared Erlenmeyer flask. To each sample 0.5 gm. of calcium carbonate was added, and enough 95 per cent redistilled alcohol to bring the concentration to 80 per cent of alcohol. Then the samples were boiled for 15 minutes. Finally, the sample flasks were tightly corked, using corks covered with lead foil.

2. *Extraction.*—Koch's method was used with a modification in the case of the water extraction. Here 1000 cc. of water was added to the dry and finely ground material from the ether extraction, and the mixture allowed to extract on the steam bath at 90° C. for 12 hours. By this modification quantitative losses of material were avoided. At the completion of the final alcohol extraction two fractions (one insoluble and the other soluble) were obtained.

3. *Separation.*—The soluble fraction contained much chlorophyll and some lipoid material. These were separated from the soluble nitrogen and carbohydrate compounds by shaking the liquid with several amounts of anhydrous ether. By the addition of the ether to the liquid and then diluting with water a clear separation was obtained. Any tendency to emulsify was prevented by a few additional drops of alcohol. The lipoid fraction, containing the chlorophyll, was drawn off and discarded. This fraction contained small

amounts of water in solution, and when analyzed for reducing substances gave amounts which were considered negligible.

4. *Estimation.*—The water and alcohol fraction which remained after the separation with ether was concentrated in vacuo at 70° C., and with the vacuum gauge indicating 20 inches, to a 500 cc. volume. Of this, a 200 cc. aliquot was used for the determination of reducing substances. The remainder was kept in reserve, and from it determinations of amino acids and soluble nitrogen were made as desired.

5. *Reducing substances.*—The 200 cc. aliquot was treated with neutral lead acetate, filtered, and the excess lead removed by the addition of potassium oxalate. On filtering again the filtrate and washings were made up to 500 cc. volume, and 50 cc. aliquots used for the determination of reducing substances. The SHAFFER-HARTMAN (21) iodometric method for the determination of small amounts of sugar was used in all carbohydrate determinations. All determinations were made in duplicate or triplicate.

6. *Total reducing substances after hydrolysis.*—An aliquot of 50 cc. of the deleaded extract was poured into a 110 cc. sugar flask and 10 cc. of concentrated hydrochloric acid added. The solution was allowed to stand at room temperature for 18 hours; then it was made up to volume and 50 cc. duplicate portions estimated for sugars.

7. *Amino nitrogen.*—Determinations were made on the liquid obtained from the ether separation, using the micro Van Slyke apparatus. The volume of the sample used was 2 cc. and the time of reaction five minutes.

8. *Nitrate nitrogen.*—The method suggested by STROWD (22) was used in the determination of nitrate nitrogen. In order to test the accuracy of this method when used with plant extracts a series of determinations was made. Varying amounts of potassium nitrate were added to 10 cc. portions of the extract from the ether separation, and the following results were obtained:

Potassium nitrate added in gm.	Nitrate nitrogen recovered in percentage
0.01	91.2
0.05	94.9
0.10	95.2
0.10	93.1

On further trial it was found that the addition of asparagine to the extract introduced a large error, an observation also noted by BURRELL and PHILLIPS (3); therefore, in order to determine the amounts of ammonia and amid nitrogen present in the extract, PHILLIP's method (19) was employed. Several extracts were used and a volume of 10 cc. in each case.

Number of extract	Ammonia nitrogen (mg.)	Amid nitrogen (mg.)
1	0.14	0.13
2	0.01	0.15
3	0.05	0.02

Thus, since no ammonia and amid nitrogen determinations were made on all the extracts, the results given as nitrate nitrogen cannot be considered as within the limits of error.

9. *Total nitrogen.*—The Kjeldahl method with the nitrate modification (18) was used, and the determinations were made on the dry moisture samples.

10. *Total insoluble nitrogen.*—Aliquots of the dry insoluble residue remaining after the final extraction were analyzed for nitrogen by the Kjeldahl-Gunning-Arnold method, using 0.7–2 gm. of the finely ground material.

11. *Total soluble nitrogen.*—The difference between the total nitrogen and the total insoluble nitrogen was considered as expressing the total soluble nitrogen.

12. *Total hydrolyzable polysaccharides.*—Direct acid hydrolysis was applied to 2.5–3 gm. of the dry insoluble extracted portion (18). All carbohydrates were expressed as invert sugar.

During the course of the analyses it became necessary to transport part of the samples from Chicago to Yonkers, New York. These samples were washed quantitatively, using small portions of alcohol, into pint jars having aluminum screw tops. A cork washer was placed in the screw top of each jar and melted paraffin poured on it until the cork became impregnated. This was allowed to partially solidify and then the top was screwed on the jar, producing a seal which quite adequately prevented loss of extract.

Experimentation

GROWTH.—*Xanthium* plants were grown under varying combinations of temperature and length of day. Observations were made

to determine the first indications of staminate flower buds, and the time from planting to flower formation calculated. The first staminate flower cluster appears in the apical region of the stem, and when the tetrad stage is reached, small protuberances are plainly visible under a dissecting microscope. In every case an average of observations from several plants was calculated, and by this means individual variation was minimized.

TABLE I

HIGH-TEMPERATURE SHORT-DAY CONDITIONS

EXPERIMENT NO.	VEGETATIVE STAGE	AVERAGE POSSIBLE SUNLIGHT IN HOURS	AVERAGE WEEKLY TEMPERATURES	
			Maximum °F.	Minimum °F.
III.........	Jan. 23–Feb. 7 (15 days)	9.7–10.4 (Average 10)	77.2 78.8	68.2 71.7
IV.........	Feb. 24–Mar. 10 (15 days)	10.0–11.9 (Average 11.5)	79.7 74.0	68.2 68.5
VII........	Mar. 12–26 (14 days)	11.8–12.4 (Average 12.1)	81.8 77.5	67.7 69.8
VIII.......	Mar. 22–Apr. 5 (14 days)	12.2–13.1 (Average 12.6)	78.6 76.7	65.8 70.6
XX........	May 20–June 2 (13 days)	10	83.7 90.5	70.2 70.2
XXIII.....	June 11–24 (13 days)	10	87.1 95.0	70.2 74.5
XXIV......	July 2–14 (12 days)	10	90.5 92.7	72.5 74.5

High-temperature short-day plants.—These were grown in the greenhouse during the months of November 1923 to March 1924 inclusive. Germination was observed from three to five days after planting. Table I gives the environmental conditions and the results of several experiments. In the cases of the final three experiments, the light duration was controlled accurately by covering the plants at the end of ten hours of daylight. Under these conditions the time of distinctive vegetative activity was very short. As a result, the plants had an average height of 11.5 cm. when measured from the soil level to the staminate bud cluster. When

allowed to set fruit a very small number of poorly developed burs resulted.

High-temperature long-day plants.—The following two experiments were carried out in the greenhouse after the natural day length had increased. In height the plants in Experiment XV averaged 25 cm. The increased day length resulted in a lengthening of the vegetative period.

TABLE II

HIGH-TEMPERATURE LONG-DAY CONDITIONS

Experiment no.	Vegetative stage	Average possible sunlight in hours	Average weekly temperatures	
			Maximum °F.	Minimum °F.
XII........	Apr. 15–May 7 (22 days)	13.4–14.3 (average 13.8)	81.5 83.7 83.7	67.5 70.2 67.0
XV........	Apr. 29–June 15 (47 days)	14.0–15.2 (average 14.6)	83.7 84.5 83.7 83.5 89.7 84.7 86.1	67.5 70.0 70.1 67.7 71.7 70.2 70.2

In addition to these three experiments, seeds were planted in shallow 4-inch pots and exposed to 24-hour illumination in the specially constructed and conditioned room used for such experiments, at the Boyce Thompson Institute. The plants were exposed to very uniform conditions of temperature and humidity, the temperature rarely showing a variance of one degree above or below 78° F., and the humidity being maintained at 86 per cent. The seeds were planted October 8, 1924; germination was observed October 11; and on November 19, when the experiment was discontinued, no signs of staminate flower buds could be found. Seven plants had reached an average height of 40.5 cm. These plants thus were still actively vegetative forty-two days after planting. Low-temperature short-day plants were started from seed November 20, 1923; germination was observed on November 26; and staminate flower buds noted on March 15, 1924. This made a total of 116 days during which the plants were actively vegetative. The average height of the plants

on March 15 was 51 cm., and cross-sections of their stems showed the presence of much strengthening tissue. Fig. 1 indicates the conditions of temperature and possible sunlight during that period. The length of the vegetative stage in these plants was comparable with that of the low-temperature long-day plants.

Low-temperature long-day plants.—The following three experiments give an indication of the reaction of *Xanthium* to this combination of environmental factors.

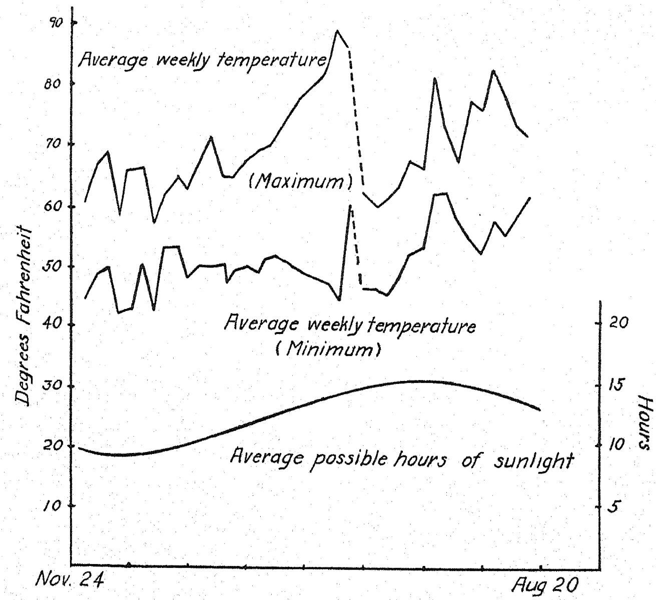

FIG. 1.—Temperature and average possible hours of sunlight from November 24, 1923 to August 20, 1924.

Experiment III.—Seeds were planted in the warm house on January 23, 1924; transferred to the cool house on February 2, and finally transplanted in the garden on May 2. Staminate flower buds were observed on August 7, thus making a total of 197 days for the active vegetative stage.

Experiment XII.—Seeds were planted in the warm house on April 15, and the plants transplanted in the garden May 2. Stami-

nate flower buds were observed on August 7, making a total of 114 days for active vegetation.

Experiment XXI.—Seeds were planted in the garden on May 20, and staminate flower buds were observed August 20, making a total of 92 days for the vegetative stage.

Fig. 1 gives a record of the temperature maxima and minima, and also the average possible hours of sunlight during the portion of the year covered by the experiments.

Analyses[1]

Following the growth experiments, an attempt was made to secure some information as to the metabolic conditions within the

TABLE III: SERIES A

HIGH-TEMPERATURE SHORT-DAY PLANTS; PERCENTAGE DRY WEIGHT BASIS

TIME IN DAYS	REDUCING SUBSTANCES	HYDROLYZABLE POLYSACCHARIDES
7	0.34	8.78
8	0.41	7.89
9	0.59	9.10
10	0.54	9.88
11	0.93	10.08
12	1.06	10.31
13	0.95	10.44
14	0.99	12.97
15	....	14.14

plants as they approached flowering, and especially when they were subjected to conditions of temperature and day length, which were distinctly opposed to each other.

SERIES A.—High-temperature short-day plants were grown in the warm greenhouse in large shallow boxes. The day length was controlled to 10 hours. The temperature data are given in table I, Experiment XXIV. Sampling was begun on the seventh day after planting, and the plants were sampled daily until the sixteenth day. Staminate flower buds were noted on the twelfth day. For each

[1] The major portion of the analyses was made at the Boyce Thompson Institute for Plant Research, Yonkers, New York.

analytical sample 150–200 plants were used. On the twelfth day the plants averaged 11.5 cm. in height, and cross-sections of the stems taken near the ground gave an average diameter of 0.4 cm. The analytical results are given in tables V, VI, and VII. Distinct increases in reducing substances, hydrolyzable polysaccharides, and

TABLE IV: SERIES A

HIGH-TEMPERATURE SHORT-DAY PLANTS; PERCENTAGE DRY WEIGHT BASIS

Time in days	Total nitrogen	Soluble nitrogen	Insoluble nitrogen	Amino nitrogen
7..........	7.00	2.62	4.38	1.81
8..........	7.10	3.78	3.32	1.59
9..........	6.68	2.48	4.20	1.00
10..........	6.55	2.65	4.00	1.07
11..........	6.58	2.56	4.02	1.09
12..........	5.55	1.25	4.30	1.16
13..........	5.58	1.38	4.20	1.36
14..........	5.31	1.64	3.67	1.36
15..........	4.70	1.22	3.60	....
16..........	4.87	1.27	3.48	....

TABLE V: SERIES A

MISCELLANEOUS DATA: HIGH-TEMPERATURE SHORT-DAY PLANTS

Time in days	Moisture percentage	Soluble C/N ratio	Total C/N ratio
7...........	93.2	0.13	1.30
8...........	95.5	0.11	1.16
9...........	90.5	0.23	1.45
10...........	92.8	0.20	1.59
11...........	91.7	0.36	1.67
12...........	93.3	0.84	2.04
13...........	92.9	0.68	2.04
14...........	91.3	0.60	2.62
15...........	90.3	....	3.00

amino acids were found, while soluble nitrogen forms decreased. Both the total and the soluble carbohydrate-nitrogen ratios increased as the plants became reproductive.

SERIES B.—Plants were grown in the garden under low-temperature long-day conditions. The environmental conditions of temperature and day length are represented in fig. 1, and are those connected with Experiment XXI. Sampling was begun on July 25,

and samples were taken weekly until August 20. Staminate flower buds were observed on August 13. At the time of final sampling

TABLE VI: SERIES B

LOW-TEMPERATURE LONG-DAY PLANTS; ANALYSES OF UPPER PARTS; PERCENTAGE DRY WEIGHT BASIS

Time in weeks	Reducing substances			Reducing substances after hydrolysis			Hydrolyzable polysaccharides		
	I	II	Average	I	II	Average	I	II	Average
9	1.45	......	......	1.98	......	......	12.21	......	......
10	1.38	1.50	1.44	1.91	1.73	1.87	17.51	16.28	16.89
11	0.64	1.09	0.86	0.94	0.96	0.95	14.26	14.41	14.33
12	2.50	2.61	2.55	3.82	3.92	3.87	19.56	19.75	19.65
13	4.57	2.34	3.45	5.43	3.28	4.35	22.40	28.27	20.33

TABLE VII: SERIES B

LOW-TEMPERATURE LONG-DAY PLANTS; ANALYSES OF UPPER PARTS; PERCENTAGE DRY WEIGHT BASIS

Time in weeks	Total nitrogen	Insoluble nitrogen			Soluble nitrogen			Amino nitrogen			Nitrate nitrogen		
9	4.82	4.52	....	....	0.30	....	....	0.02	....	....	....	....	....
10	4.81	4.53	4.40	4.46	0.35	....	....	0.06	0.09	0.07	0.12	0.16	0.14
11	4.81	4.29	4.72	4.50	0.27	0.36	0.31	0.08	0.13	0.10	0.29	0.29	0.29
12	4.98	4.35	4.02	4.68	0.20	0.35	0.27	0.13	0.12	0.13	0.24	0.14	0.19
13	4.54	4.22	3.97	4.09	0.45	....	....	0.15	0.14	0.15	0.15	0.12	0.14

TABLE VIII: SERIES B

LOW-TEMPERATURE LONG-DAY PLANTS; ANALYSES OF LOWER PARTS; PERCENTAGE DRY WEIGHT BASIS

Time in weeks	Reducing substances			Reducing substances after hydrolysis			Hydrolyzable polysaccharides		
	I	II	Average	I	II	Average	I	II	Average
9	1.82	1.83	1.82	2.49	2.76	2.62	5.01	5.14	5.07
10	1.20	1.56	1.38	3.65	3.46	3.55	5.27	7.54	6.40
11	1.98	1.13	1.55	1.90	1.21	1.55	7.75	7.91	7.83
12	2.72	0.64	1.68	4.24	1.57	2.90	8.36	11.90	10.13
13	2.25	6.36	4.30	4.09	9.00	6.54	22.12	19.03	20.57

the plants averaged 94 cm. in height, and had an average stem diameter of 2.5 cm. when sectioned at the first node above the ground. The plants of this series were divided into two portions

for analysis, as the amount of material was sufficient for both duplicate samples and for analyses of tops and lower portions. The

TABLE IX: SERIES B

LOW-TEMPERATURE LONG-DAY PLANTS; ANALYSES OF LOWER PARTS; PERCENTAGE DRY WEIGHT BASIS

TIME IN WEEKS	TOTAL NITROGEN	INSOLUBLE NITROGEN			SOLUBLE NITROGEN			AMINO NITROGEN			NITRATE NITROGEN		
		I	II	Average	I	II	Average	I	II	Average	I	II	Average
9	3.13	1.18	1.42	1.30	1.95	1.80	1.87	0.06	0.06	0.06	0.10	0.21	0.15
10	3.41	1.66	1.60	1.63	1.75	1.81	1.78	0.13	0.06	0.09	0.53	0.75	0.64
11	2.50	....	1.04	....	....	1.46	....	0.05	0.10	0.07	0.64	0.53	0.58
12	2.82	1.07	1.41	1.24	1.75	1.41	1.58	0.11	0.10	0.11	0.65	0.42	0.53
13	2.13	1.11	1.09	1.10	1.02	1.04	1.03	0.73	0.26	0.49	0.10	0.11	0.10

TABLE X: SERIES B

LOW-TEMPERATURE LONG-DAY PLANTS; AVERAGED RESULTS OF TABLES VI AND VIII TO COVER ENTIRE PLANTS; PERCENTAGE DRY WEIGHT BASIS

TIME IN WEEKS	REDUCING SUBSTANCES	TOTAL REDUCING SUBSTANCES AFTER HYDROLYSIS	HYDROLYZABLE POLYSACCHARIDES
9	1.63	2.30	8.64
10	1.41	2.71	11.64
11	1.20	1.25	11.08
12	2.11	3.38	14.89
13	3.87	5.44	20.45

TABLE XI: SERIES B

LOW-TEMPERATURE LONG-DAY PLANTS; AVERAGED RESULTS OF TABLES VII AND IX TO COVER ENTIRE PLANTS; PERCENTAGE DRY WEIGHT BASIS

TIME IN WEEKS	TOTAL NITROGEN	INSOLUBLE NITROGEN	SOLUBLE NITROGEN	AMINO NITROGEN	NITRATE NITROGEN
9	3.97	2.91	1.08	0.04	0.15
10	4.11	3.04	1.06	0.08	0.39
11	3.65	2.77	0.88	0.08	0.43
12	3.90	2.96	0.92	0.12	0.36
13	3.33	2.59	0.74	0.32	0.12

point of division was chosen as the fifth node from the ground, the roots being discarded. The analytical results are given in tables VI–XII. These results show the presence of greater quantities of

reducing substances and total reducing substances after hydrolysis in the lower parts of the plants, while insoluble nitrogen forms are high in amount in the tops. Soluble nitrogen compounds are highest in the lower portions however. When the entire plant is considered, as in tables XI and XII, it is seen that carbohydrate forms increased in amount, while soluble nitrogen forms showed a distinct falling off.

TABLE XII: SERIES B

MISCELLANEOUS DATA: LOW-TEMPERATURE LONG-DAY PLANTS

Time in weeks	Moisture percentage	Soluble C/N ratio	Total C/N ratio
9	86.86	1.50	2.75
10	86.47	1.33	3.17
11	83.13	1.36	3.36
12	84.80	2.29	4.36
13	84.58	5.22	7.30

In order to allow comparison between the ratios in the two series, the total reducing substances after hydrolysis as estimated in Series B were not included in the calculations.

Seedling stages

A study of the effect of temperature on metabolism of *Xanthium* during the seedling stage was made. Seeds were germinated at two widely differing temperatures and analyses made.

MICROCHEMICAL TESTS.—Seeds were germinated in Petri dishes kept at 95° and 68° F. on moistened filter paper. Microchemical tests were made daily for the presence of fats, carbohydrates, protein, and amino acids in both cotyledons and hypocotyls. The tests were discontinued when Sudan III no longer gave a positive test for fats in the cotyledons. No tests for starch or glucose were obtained in the dry seed, although an amorphous precipitate resembling copper oxide was obtained with the Flückiger test. After twenty-four hours' germination at the higher temperature this reduction became very marked, and the presence of glycerine was demonstrated by using the acrolein test. It is possible, therefore, that this reduction was caused by intermediate products in the process of transformation from fats to carbohydrates. At the lower

temperature a marked development of pigment and a definite accumulation of reducing carbohydrates took place in the first few centimeters of the hypocotyl. These results were in direct contrast with the higher temperature seedlings, where growth was very rapid and the accumulation of carbohydrates was prevented. When the lower temperature seedlings were allowed to grow, they developed all the characteristics of the low temperature plants. An estimation of the varying proportions of metabolic materials in cotyledons and hypocotyls is given in table XIII.

TABLE XIII

MICROCHEMICAL ANALYSES OF SEEDLINGS

Time of germination in hours	Fats		Carbohydrates				Proteins					
	Sudan III		Flückiger test		Osazone test		IKI test		Millon's test		Xanthoproteic test	
	95° F.	68° F.	95° F.	68° F.	95° F.	68° F.	95° F.	68° F.	95° F.	68° F.	95° F.	68° F.
24......	***	***	.....	.....	.....	.....	***	***	***	***	***	***
48......	**	***	.....	**	.....	**	***	***	***	***	***	***
80......	*	***	*	**	.....	***	**	***	**	***	***	***
96......	*	***	**	**	*	***	**	***	**	***	**	***
120......	.....	**	**	***	**	***	**	***	**	***	**	***

MACROCHEMICAL TESTS.—*Xanthium* seeds were germinated in constant temperature ovens at 90° and 72° F., on filter paper kept moist by keeping it in contact with wet sand. Sampling was begun when the hypocotyl radicles were 3 inches in length, and 500–1000 seedlings composed each sample. The hypocotyl radicles were severed from the cotyledons and analyzed separately. The material was frozen, ground, and preserved as already described in the case of the plant samples. All analytical results are the averages of at least two determinations. The amount of material was too small to allow the use of duplicate samples. From the results given in tables XIV and XV, the seedlings grown at the lower temperature gave on analysis much larger amounts of total reducing substances after hydrolysis, hydrolyzable polysaccharides and amino acids, results which agree with those already indicated by microchemical tests.

DICKSON, ECKERSON, and LINK (4), working with wheat seedlings, found that at low soil temperatures the rate of starch hydroly-

sis is more rapid than that of protein food reserves. This makes the young plants practically carbohydrate-high, and as a result they have cell walls thicker and more resistant to the invasion of fungus

TABLE XIV

EFFECT OF TEMPERATURE ON GERMINATION OF XANTHIUM SEEDLINGS

TEMPERATURE (°F.)	REDUCING SUBSTANCES	TOTAL REDUCING SUBSTANCES	HYDROLYZABLE POLYSACCHARIDES
	Cotyledons		
90	0.06	2.28	0.13
72	0.64	4.90	0.14
	Hypocotyls		
90	9.55	12.01	2.34
72	7.51	16.63	4.29

TABLE XV

EFFECT OF TEMPERATURE ON GERMINATION OF XANTHIUM SEEDLINGS

TEMPERATURE (°F.)	SOLUBLE NITROGEN	INSOLUBLE NITROGEN	AMINO NITROGEN
	Cotyledons		
90	2.75	2.68	0.90
72	2.11	4.07	1.57
	Hypocotyls		
90	4.25	6.98	1.71
72	4.41	3.13	2.11

hyphae. In the case of *Xanthium* the lower temperatures bring about an earlier hydrolysis of the carbohydrate forming reserves, and a slower hydrolysis of the protein reserves.

Discussion

Recently much stress has been placed on relative day length as one of the most important formative factors to which a plant is subjected by its environment. This phenomenon, however, is only one of many which influence a plant during its growth period, and therefore humidity, nutrient supply, and temperature must greatly

modify its importance as a formative factor. The great complexity of environmental combinations in nature would lead us to believe that, while at one time one specific factor may be the cause of the inhibition or acceleration of a certain formative change, under a

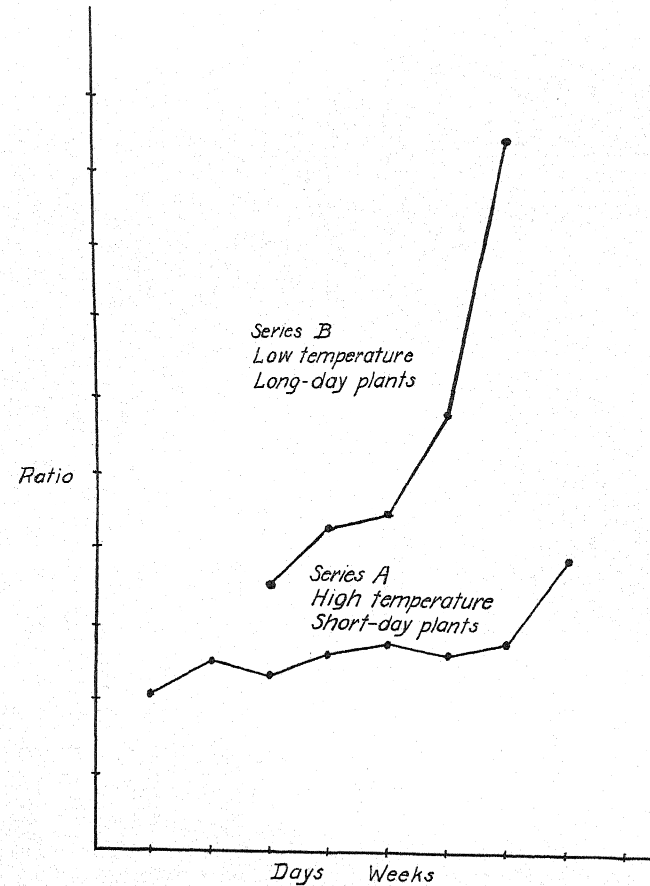

FIG. 2.—Ratio of total carbohydrates to total nitrogen

different set of conditions another factor may assume the same rôle. BLACKMAN (2) has emphasized the theory of limiting factors which control photosynthesis, and among his factors he has given temperature a prominent place. In a similar manner relative day length may possibly act as a deciding factor under certain combinations of

conditions, and thus inhibit or accelerate vegetative activity. Evidence is also gradually accumulating to show that the length of the vigorously vegetative period of certain plants is affected to a like degree by temperature and day length. Such seems to be the case with *Xanthium*. Further experimentation under more accurately controlled environmental conditions may be expected to show that, even as specific day lengths are limiting for vegetative activity with certain plants, so, given certain environmental combinations, specific temperatures may be found to substitute for relative day length in the development of flower primordia.

It is quite generally accepted that a change in the growth habit, such as that from vegetation to reproduction, is accompanied by changes in the metabolic equilibria within the plant. At present, the only means of studying these changes is by the analytical method. Thus, if a series of analytical results can be obtained which shows a decided tendency in any one direction, some criterion of the metabolic state may be suggested. In *Xanthium*, comparisons between total carbohydrates and total nitrogen (fig. 2), and soluble carbohydrates and soluble nitrogen (fig. 3) show the ratios to be distinctly ascending as flower primordia are formed under the

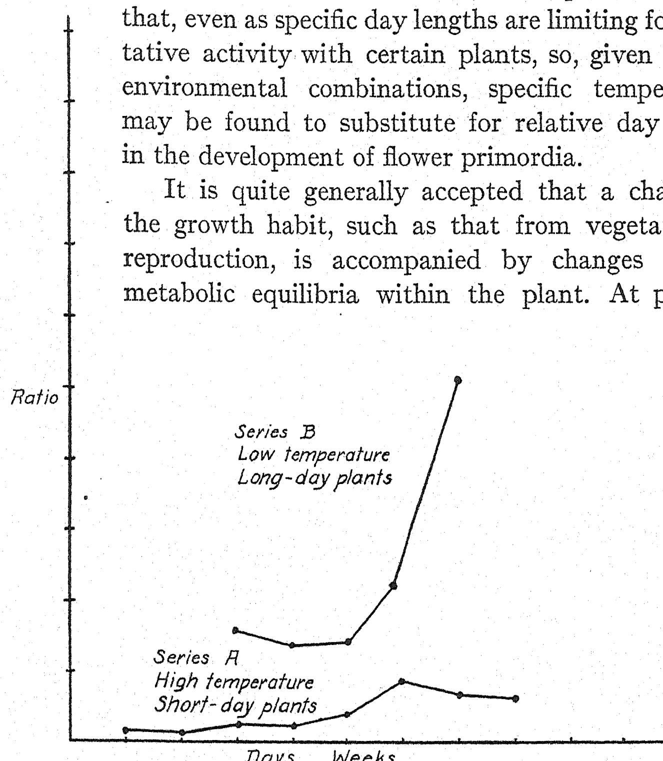

FIG. 3.—Ratio of soluble carbohydrates to soluble nitrogen

influence of both sets of conditions. This is in accord with the work of KRAUS and KRAYBILL and others. Recently NIGHTINGALE has stressed the ratio of carbohydrates to insoluble nitrogen as being more indicative of metabolic conditions. Following his suggestion, the ratios of total carbohydrates to insoluble nitrogen (fig. 4), and

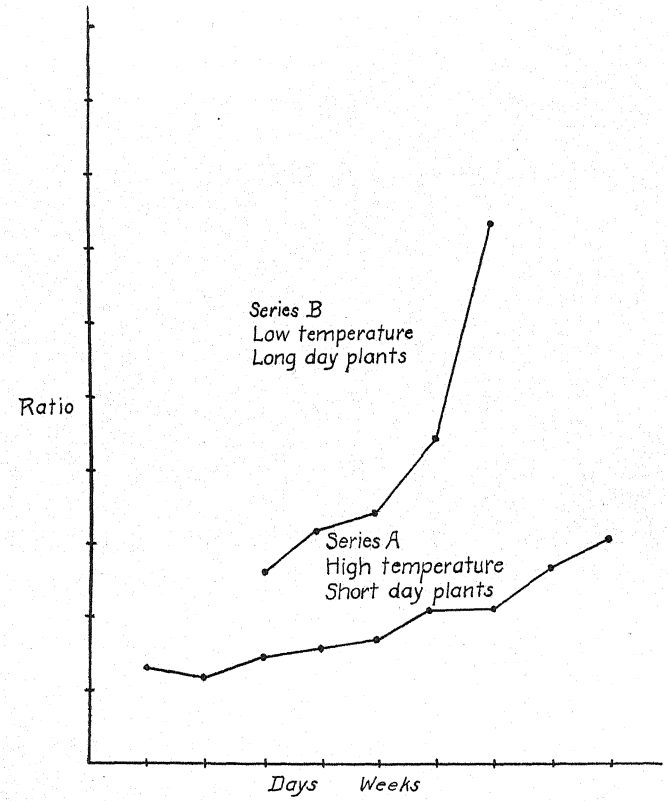

FIG. 4.—Ratio of total carbohydrates to insoluble nitrogen

soluble carbohydrates to insoluble nitrogen (fig. 5) are given for *Xanthium*.

The careful study of material grown under a wider range of conditions is considered desirable before any conclusion as to the significance of such comparisons can be of value. It should be noted that the results given by Series B cover much shorter portions of

the life of the plant than do those of Series A. If weeks and days are equated, the ninth week corresponds closely to the ninth day, and so the actual portion of the history of the plant covered by both might be considered as being comparable. While it must be borne in mind that no single ratio can at present be assigned a causative rôle in the formation of flower primordia, it is worthy of note that in the two series ascending carbohydrate nitrogen ratios were noted, even though the total lengths of the vegetative periods contrasted greatly. These comparable results justify the conclusion that while *Xanthium* is very suceptible to temperature and relative day length, when reproduction does begin the same sort of metabolic conditions occur when the interrelation of carbohydrate and nitrogen compounds are considered.

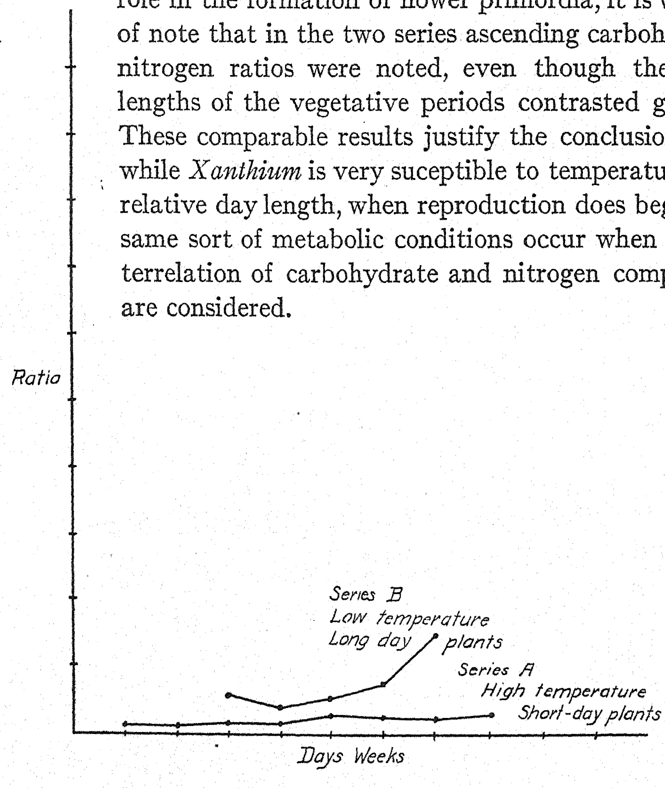

FIG. 5.—Ratio of soluble carbohydrates to insoluble nitrogen

By some, conditions during the germinative stage have been considered as important from the standpoint of their effect on the after history of the plant. It has been suggested by WALSTER that high temperature, coupled with high nitrogen supply, shifts the metabolic equilibria toward excessive vegetation, and inhibits the tendency toward normal vegetation. This must be counteracted by some other factor during the vegetative stage if reproduction is to

be expected. The analytical results with *Xanthium* seedlings seem to throw some light on such a tendency. The low temperature seedlings contain greater amounts of higher carbohydrate compounds, thus showing a tendency toward the formation of mechanical and other tissues found in low temperature plants. This accumulation of carbohydrate compounds was no doubt facilitated by the low temperature conditions which resulted in a lower respiratory rate. There can be no doubt but that the strongly vegetative characteristics of the low temperature plants received their start by low temperature conditions during germination and early seedling stages. It is doubtful, however, whether a tendency toward vegetation or reproduction due to early temperature conditions can account for the marked reactions to both temperature and day length during the later stages of the actively vegetative period of *Xanthium*.

Summary

1. Growth experiments and chemical analyses were made with plants of *Xanthium pennsylvanicum*, grown under known conditions of temperature and relative day length.

2. Temperature was found to be a determining factor in influencing the time of flower primordia formation. This temperature effect, however, was closely associated with a response to relative day length.

3. The interrelation of day length and temperature was shown to be as follows: (1) high-temperature short-day plants gave indications of flowering 12–15 days after planting; (2) high-temperature long-day plants were found to flower 47 days after planting; (3) low-temperature short-day plants vegetated actively for 116 days before staminate flower buds were produced; (4) low-temperature long-day plants remained actively vegetative for a minimum of 92 days.

4. Chemical analyses of high-temperature short-day and low-temperature long-day plants resulted in ascending carbohydrate nitrogen ratios in both cases as the plants approached flower primordia formation. A marked difference in the magnitude of the ratios was noted.

5. Chemical analyses of seedlings germinated under high and

low temperature conditions showed a marked accumulation of reducing sugars and condensed sugar forms in the low temperature hypocotyl radicles. This accumulation suggests a physiological predetermination influencing the initial stages of growth.

The writer gratefully acknowledges his indebtedness to Professor C. A. SHULL for his helpful advice and encouragement at all times; to Dr. SOPHIA ECKERSON and Dr. H. R. KRAYBILL for their kindly aid and criticism; and to Dr. WILLIAM CROCKER for placing at his disposal the excellent laboratory facilities of the Boyce Thompson Institute for Plant Research.

AGRICULTURAL EXPERIMENT STATION
KINGSTON, R.I.

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STUDIES IN THE GENUS BIDENS. VII

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 344

EARL EDWARD SHERFF

(WITH PLATES I–IV)

Bidens Moorei, sp. nov. (Pl. 1)—Herba perennis, glabra, 3–5 dm. alta, caule adscendente et ramoso; ramis plus minusve glaucescentibus, monocephalicis, gracilibus, supra nudis, infra foliis parvulis foliosis. Folia sessilia, lanceolata vel cuneato-oblanceolata, integra vel versus apicem irregulariter dentata, carnosa, 1–6 cm. longa. Capitula terminalia, longe pedunculata, ligulata, 4–5 cm. lata. Involucrum squamis duplici serie dispositis; exterioribus circiter 8, ovatis vel ovato-lanceolatis, 6–8 mm. longis; interioribus lanceolatis, membranaceis, striatis, 1.2–1.4 cm. longis. Flores ligulati circiter 8, flavidi, ligula lineares, striati, apice dentati 2–2.6 cm. longi. Achaenia linearia, plana vel subtetragona, adscendenti-ciliata et -hispida, corpore 0.8–1.5 cm. longa et 1–1.2 mm. lata, bi- (saepe imperfecte tri- vel quadri-) aristata aristis retrorsum hamosis, usque ad 5 mm. longis.

John Gossweiler 3339, in woods, Angola, April, 1906 (type in Herb. Brit. Mus.); *idem* 2467, in sandy, herb-grown woods, January 5, 1906; *H. Baum* (Reise nach Südwest-Africa) 708, at altitude of 1275 m., sandy ground among low bushes, along the Longa River ("oberhalb des Quiriri," "oberhalb Ninnescra"), Angola, February 5, 1900 (Herb. Berl., Herb. Brit. Mus.).

O. HOFFMANN, in his original determination of BAUM's plant just cited, treated it as a new species. Whether he did so because at that time he was unfamiliar with *Bidens andongensis* Hiern, or because, although familiar with HIERN's species, he felt BAUM's plant to be distinct, I am unable to say. However, he later (Kunene-Sambesi Exped. 420. 1903) referred the BAUM specimen to *Bidens andongensis* Hiern. Since the main differences lay in the size and shape of the involucres, also the number and shape and proportionate size of the exterior involucral bracts, one might indeed have suspected that BAUM had collected merely an atypical and less robust form of *B. andongensis*. The various specimens sent in more recently by GOSSWEILER to the Herbarium of the British Museum, however, show similar peculiarities as to these characters, thus strengthening the belief that we have to do with a distinct species.

The species is here named in honor of SPENCER L. MOORE of the Herbarium of the British Museum of Natural History, who has done much admirable work upon the African species of *Bidens*, and who, independently of myself, had likewise interpreted the above GOSSWEILER plants as typifying a new species.

BIDENS MOOREI **verrucosa**, var. nov.—A specie differt achaeniis verrucatis; his plerumque 6–8 aristatis, saepe praeterea 4–10 laevibus acutis aristulis coronatis.

John Gossweiler 3021, Angola, April, 1906 (type in Herb. Brit. Mus.).

A form so peculiar in fruit characters that it is perhaps best treated for the present as a variety. Various considerations indicate no basis for regarding it as a separate species. The achenes are minutely warty and 5–8-aristate (commonly with 3 long aristae and 2–5 short ones, with frequently 4–10 additional rudimentary, smooth, sharp aristae completing a crown). The variety is seen to be comparable, in its relation to the species proper, with the var. *verrucifera* Moore of *Bidens crocea* Welw. In its large number of aristae it makes a singular approach to *Ericentrodea mirabilis* (Sherff) Blake and Sherff, a plant that differs widely, however, in other respects.[1]

Ericentrodea Blake and Sherff, gen. nov.—Fructices (et herbae?) scandentes, foliis oppositis, petiolatis, ternatis vel biternatim divisis, coriaceis; capitulis cymoso-paniculatis, discoideis vel radiatis, flavis; involucro duplici ut in Bidente, bracteis exterioribus parvis, herbaceis, interioribus submembranaceis, lineatis; receptaculo subplano; paleis subplanis, membranaceis, lineatis; ligulis (si praesentibus) pistillatis, fertilibus; disci floribus hermaphroditis, corollis tubulosis, tubo tenui, gutture infundibuliformi, limbo breviter 5-denticulato; antherarum basibus cordato-sagittatis et appendicibus ovatis terminalibus; stylo exserto, ramis brevibus, ramorum appendicibus triangularibus, acuminatis, papillatis; achaeniis valde obcompressis, corpore obovato manifeste vel obsolete 2-alato, ad marginem lobulatum grosse ciliato, ad apicem in collare vel cervicem brevem angustato; aristis fragilibus, circ. 6–15 (3–"8" supra utrumque achaeniorum angulorum et inter se plerumque basi plus minusve connatis, utroque achaeniorum laterum interdum 2 vel 3 aristis brevioribus intermediis armato.

Type species *Narvalina corazonensis* Hieron.

BIDENS CHINENSIS (L.) Willd. var. ABYSSINICA (Schz. Bip.) O. E. Schulz, Engler Bot. Jahrb. **50** (Supplem.) : 180. 1914; *B. abyssinica* Schz. Bip., Walpers Repert. **6**:167. 1846–1847: *B. abyssinica* var. *quadriaristata* Hochst. *ex* Schweinfurth Beitr. Fl. Aethiop. **1**:142. 1867; *B. quadriseta* Hochst. *ex* Oliver, Fl. Trop. Afr. **3**:393. 1877: *B. abyssinica* var. *incisifolia* Hochst. *ex* Oliv. *l.c.;*

[1] *Bidens mirabilis* Sherff, BOT. GAZ. **61**:496. 1916.—In a recent paper (Jour. Wash. Acad. Sci. **13**:104. 1923), S. F. BLAKE and I have joined in creating the new generic name *Ericentrodea* for this and two other species. The generic description, there given in English, is here repeated in Latin to satisfy the requirements of the Vienna Code of Nomenclature:

B. lasiocarpa O. E. Schulz, Engler Bot. Jahrb. 50 (Supplem.):185. 1914.

Some of SCHIMPER's original specimens, on which were founded the names *Bidens abyssinica*, *B. abyssinica* var. *quadriaristata*, and *B. quadriseta*, are densely hairy forms with tripartite leaves and conspicuously large fruiting heads. In leaf characters they might be confused with *B. pilosa* L.; their achenes and involucral bracts, however, are very distinct. Some cotypes of "*B. abyssinica*" (for example, in Herb. Brit. Mus.) show leaves more incisely toothed or lobed and approaching more or less clearly those of *B. chinensis* (L.) Willd. A diminutive specimen collected by SCHWEINFURTH and RIVA (no. 804, in Herb. Boiss.) has the leaves glabrous and bipinnate as in *B. bipinnata* L. The fruit characters sometimes seem much too distinctive to warrant giving "*B. abyssinica*" a varietal rank under *B. chinensis* as proposed by O. E. SCHULZ, but the great number of intergradations observed in various herbarium specimens appears to compel such a course. It may be observed, however, that the variety is to be separated from the species not so much by its hairiness, which SCHULZ gives as the distinguishing character, as by its longer achenes, the outer ones often densely setose.

The varietal name *incisifolia* was given originally to specimens of *Schimper* 2328, described (OLIVER, *l.c.*) as having "rather more deeply cut leaf-lobes." The authentic sheet of this number at Kew, however, marked "Fl. Afr. Trop. iii p. 393," has none of its leaves incisely toothed to a noticeable degree.

In proposing as a new species his *Bidens lasiocarpa*, SCHULZ gave emphasis (in key, *l.c.* 187) to the outer achenes being more or less recurved and commonly very densely hirtous, but this character is present equally well in the type material of the var. *abyssinica*, and seems to have been overlooked by him. Moreover, *B. lasiocarpa* has long achenes, as in the type material of the var. *abyssinica*, and, while differing in the variable character of leaf hairiness, is too closely connected by intermediate forms in the herbaria to rank as a separate species.

BIDENS GRANDIS Sherff, BOT. GAZ. 59:309. 1915; *Coreopsis lineata* Klatt, Ann. Naturh. Hofmus. Wien 7:103. 1892; *C. speciosa* Hiern, Cat. Welw. Pl. 1^{III}:585. 1898.

In a previous paper (SHERFF, *l.c.*) this species was transferred from *Coreopsis* to *Bidens*, being named entirely anew because there was already the valid *Bidens speciosa* Gardner. Recently, on studying the large collections of the Berlin Herbarium, I found two sheets of KLATT's type specimens of his *Coreopsis lineata* (*Alex von Mechow* 131, Pungo Andongo, Angola, January–April, 1879). These not only came from one of the localities where *Bidens grandis* originally was collected, but match the type material of *B. grandis* (Herb. Brit. Mus.) well. The existence of *Bidens lineata* Sherff (BOT. GAZ. 76:84. 1923) fortunately precludes the necessity of going back to take up KLATT's earlier published trivial name for *B. grandis*.

Bidens cylindrica, sp. nov.—Herba gracilis, erecta, 4–7 dm. alta, caule tetragono, glabro, ramoso, circ. 2–3 mm. diam. Folia petiolata petiolis tenuibus 1–5 cm. longis, petiolo adjecto principalia 5–13 cm. longa, pinnatim 3–5-partita foliolis lanceolatis vel lateralibus ovatis, membranacea, serrata dentibus acriter apiculatis, margine ciliata, faciebus glabrata vel sparsissime adpresso-pilosa. Capitula ramos tenues plerumque nudos usque ad 13 cm. longos terminantia, plerumque subradiata, ad anthesin 4–5 (rarius –10) mm. alta et 3.5–4.5 (rarius –7) mm. lata, saepe cylindrica. Involucri bracteae exteriores 6–8, lineares, versus apicem saepius angustatae, apice acutae, margine piloso-ciliatae, faciebus glabrae vel sparsim hispidae, 3–8 mm. longae et 0.3–1 mm. latae; interiores lanceolatae, apice subacutae, paulo vel interdum multo longiores. Flores ligulati rudimentarii, albidi vel rosaceo-albidi, oblongo-obovati, apice subtruncati et circ. 3-dentati. Achaenia 6–12, linearia, tetragona, atra, glabra vel exteriora saepe hispida, corpore 1.2–1.6 mm. longa et 0.5–0.8 mm. lata, bi- vel triaristata aristis moderate tenuibus retrorsum hamosis 2.5–4 mm. longis.

Specimens examined.—*Menyhart* 1110, not abundant, in shady places at St. Joseph, Boruma ("Boroma"), Northern Zambesia, Rhodesia, April, 1892 (type in Herb. Univ. Vienna); *Anon.*, cult. in Bot. Gard. Vienna *ex sem. Menyhartii* 1110 (2 sheets, Herb. Univ. Vienna); *Aug. Chevalier* 2816, along the Schari River, Tschad Lake district, Central Africa, October 26, 1899 (Herb. Berl.).

The CHEVALIER specimen listed here had been determined by O. E. SCHULZ as his *Bidens Engleri*, and in fact was cited by him for that species at the time of his original description (Engler Bot. Jahrb. 50 (Supplem.):186. 1914). Recently, however, I have been privileged to study not only SCHULZ's type of *B. Engleri* (Herb. Berl.), but also excellent sheets of duplicate material (Herb. Boiss.; Herb. Mus. Vienna, etc.). These all agree in having plants of low stature (2–3 dm. high), the leaves practically all undivided, the heads discoid; the exterior involucral bracts only about 3 or 4, spatulate, serrulate-ciliate, 1–2 mm. long; the inner bracts 2–5 times as long, often rounded at apex; the achenes all glabrous, parallel-sided through most of their length and thus *oblong*-linear, compressed, about 1 mm. wide. From this material the CHEVALIER and MENYHART specimens are seen to differ sharply as follows: Height 4–7 dm., leaves 3–5-parted, heads subradiate; the exterior involucral bracts 6–8, linear and mostly narrowed above, ciliate with slender hairs instead of with minute serrulations, 3–8 mm. long; the inner bracts mainly 1.1–2 times as long, subacute at apex; the outer achenes frequently hairy, all narrower and gradually attenuate upward, distinctly tetragonal, only 0.5–0.8 mm. wide. In my opinion the CHEVA-

LIER and MENYHART specimens are to be considered as specifically distinct from *B. Engleri*. They are named *B. cylindrica* in allusion to the shape of the flowering heads, which, in the two cultivated specimens studied, are notably cylindric.

BIDENS GRACILIOR (O. Hoffm.) Sherff, BOT. GAZ. **76**:84. 1923; *Coreopsis exaristata* var. *gracilior* O. Hoffm., Engler Pflanzenw. Ost-Afr. **C**:414. 1895.

The two sheets of material (*Stuhlmann* 6403 and 7584) cited by OTTO HOFFMANN for his var. *gracilior* differ from his species proper in having the leaves less glandular-pubescent or sometimes even glabrate, their divisions finer and much more acute or even acuminate, the involucre less pubescent and the achenes not being only 3–4 mm. long with all except the innermost ones oblanceolate, but being rather 4.5–6 mm. long and all linear. The important achenial differences seem to have been overlooked entirely by HOFFMANN.[2] A specimen collected later by *Holtz* (no. 406, Dar-es-Salaam, German East Africa, December 6, 1901) in the immediate vicinity of the type locality, Usaramo,[3] agrees fairly well with the type as to foliage and involucre, and has likewise the longer achenes. These important characters of foliage and achenes appear to entitle HOFFMANN's variety to separate specific rank. The following description is drawn from the STUHLMANN and HOLTZ plants:

BIDENS GRACILIOR (O. Hoffm.) Sherff, descript. amplific.—Herba perennis, gracilis, erecta, 4–7 dm. alta, caule angulato, ramoso. Folia petiolata petiolis tenuibus 0.5–2 cm. longis, petiolo adjecto 3.5–8 cm. longa, circumambitu triangulato-ovata, 1–2-pinnati-partita, segmentis ovato-lanceolatis vel lanceolato-linearibus, membranaceis, atro-punctulatis, dentibus acerrime indurato-apiculatis. Capitula tenuiter pedunculata pedunculis 4–16 cm. longa, radiata, pansa ad anthesin 2.5–4 cm. lata et 6–9 mm. alta. Involucrum glabratum vel moderate pubescens, bracteis exterioribus 6–8, linearibus, acriter cartilagineo-apiculatis, 4.5–6.5 mm. longis, quam interioribus lanceolatis plerumque paulo brevioribus. Flores ligulati circ. 8, lutei, ligula elliptico-oblanceolati, apice obscure denticulati, 1.2–1.8 cm. longi. Achaenia linearia, obcompressa, atra, supra erecto-setosa, exalata, 4.5–6 mm. longa et 0.6–1 mm. lata,

[2] Many mature achenes on the type, *Stuhlmann* 6403 (Herb. Berl.) have 1 or 2 minute aristae (0.2–0.3 mm. long) and several have these aristae retrorsely barbed with 1 or 2 barbs. Had HOFFMANN noticed this character he certainly would have remarked upon it, probably even referring his specimen not to *Coreopsis* but to *Bidens*, since with him the retrorse-barb character was considered diagnostic of the genus *Bidens* (*cf.* SHERFF, BOT. GAZ. **59**:305–308. 1915).

[3] Spelled also Uzaramo (Century Atlas, 1899).

apice calva vel breviter biaristata aristis nudis vel retrorsum 1- vel 2-hamosis et tantum circ. 0.2–0.3 mm. longis.

BIDENS ANDICOLA H.B.K., Nov. Gen. et Sp. 4:237 (186). 1820; *B. andicola* H.B.K. vars. *normalis* and *heterophylla* O. Kuntze, Rev. Gen. Pl. 3^II:136. 1898; *B. fruticulosa* Mey. and Walp., Nov. Act. Nat. Cur. 19 Supplem. I. 271. 1843.

Descript. amplific.—Herba perennis, semi-procumbens vel etiam erecta, valde hispido-pubescens vel fere glabra, ramosa, 2–8 dm. alta, caulibus parce angulatis. Folia 1–7 cm. longa, valde polymorpha; nunc indivisa, ovata, serrata, sessilia vel alato-petiolata, ad apicem obtusa vel subacuta; nunc tripartita vel 1–3-pinnata foliolis ovatis vel lanceolatis vel linearibus et ad apicem sensim vel abrupte apiculatis. Capitula ramos terminantia, longe pedunculata, radiata; pansa ad anthesin 2–4 vel rarius etiam usque ad 5.5 cm. lata, 0.7–1.4 cm. alta. Involucrum perspicue hispidum, bracteis exterioribus 8–10, lanceolatis vel lineari-oblongis, ciliatis, supra saepe glabratis, apice plerumque obtusis, quam interioribus lanceolatis dense hispidis plerumque multo brevioribus. Flores ligulati saepius 8, lutei, ligula elliptico-oblanceolati, apice plerumque minute 3-denticulati, 1.2–2.5 cm. longi. Achaenia tenuiter linearia, inferne sensim attenuata, obcompresso-quadrangularia, sulcata, supra plus minusve erecto-hispida, fusco-nigra, corpore 0.7–1.4 cm. longa et 0.4–1 mm. lata et paleas demum superantia, apice bi- (vel pauca tri-) aristata, aristis tenuibus, brunneo-stramineis vel rubescentibus, retrorsum hamosis, 1.7–3 mm. longis.

BIDENS ANDICOLA var. DECOMPOSITA O. Kuntze, *l.c.; B. macrantha* Griseb., Abhandl. Goett. 19:138. 1874; *B. grandiflora* Balb. var. *breviloba* O. Kuntze, *l.c.*—Folia 2–3-pinnatisecta, usque ad 1 dm. longa, achaeniis superne valde attenuato-elongata.

For many years the identity of the South American *Bidens andicola* has been obscured for herbarium workers by the great multiplicity of foliage forms encountered. WEDDELL, as early as 1856 (Chloris And. 1:70) described it as a polymorphous plant ("Plante polymorphe et très repande dans la chaine, mais presque exclusivement alpestre"). Later, OTTO KUNTZE, who like WEDDELL had collected in South America, commented upon the variability of the leaves ("Eine robuste Art mit einfach oder mehrfach ternatisecten Blättern, mittelgrossen gelben Strahlblüthen, ziemlich grossen Blüthenköpfen, äusseren zottig

behaarten Involucralbracteen etc., aber in Bezug auf Blatttheilung wie manche andere Bidens-Art sehr variabel"; Rev. Gen. Pl. 3^{II}:136. 1898). In herbaria the numerous foliage forms are seen to simulate corresponding forms of *B. triplinervia* H.B.K. (*B. humilis* H.B.K., *B. crithmifolia* H.B.K., etc.), and this has led often to confusion between the two species. Recently I was enabled, through the courtesy of OTTO BUCHTIEN (*cf.* SHERFF, BOT. GAZ. **76**:151. 1923), to study a great number of specimens collected by him and displaying a wide range of variation. From these (all in Herb. Field. Mus.) and many others, totalling more than two hundred specimens, the preceding descriptions are drawn. It was found that sometimes, in poorly developed material, distinction from *B. triplinervia* is apparently impossible. In well developed material, however, the distinctions are usually very definite, *B. andicola* being coarser, its thicker heads having commonly about eight instead of commonly about five rays,[4] etc. *B. andicola* has the paleae shorter than the mature achenes and this character separates it from the surprisingly similar aggregation of Mexican forms (*Purpus* 1547, 1548, 2637, 4135, 5089, 5620; *Rose* and *Painter* 6666, 7949; *Pringle* 4915; *E. W. Nelson* 3220, etc.) that in late years have passed erroneously under the name *B. daucifolia* DC. In the latter[5] the paleae are usually very blackish above and commonly surpass the mature achenes.

The type sheet of *B. fruticulosa* Mey. and Walp. is in Berlin. It bears two small specimens collected by MEYEN in April, 1831, about Tacora, Peru. The leaves are undivided, ovate to oblanceolate, and only about 1 cm. long. The heads are 6–8-ligulate. The plants match very closely a certain form found among the Buchtien plants already cited (no. 4305), and thus are seen to be merely an extreme form of *B. andicola.*

Occasionally a form of *B. andicola* is found with the leaves highly compound and the achenes strongly narrowed above, somewhat like those of *Cosmos*. If it were not for various connecting forms this would seem to be specifically distinct. KUNTZE, who himself collected specimens of it, referred at least one of them, a plant from Cochabamba, Bolivia (Herb. N.Y. Bot. Gard.) to *B. andicola*, naming it var. *decomposita*. In a careless moment he named a precisely identical form from between Cochabamba and Rio Juntas, Bolivia (Herb. N.Y. Bot. Gard.) *B. grandiflora* Balb. var. *breviloba*, although *B. grandiflora* is a Mexican species and is not known to occur in South America.

To KUNTZE's variety *decomposita* must be referred *B. macrantha* Griseb., founded on a plant by P. G. LORENTZ, no. 316, alpine pastures near Cienega, Province Tucuman, Argentina. The type is extant in good condition (Herb. Berl.). Although lacking mature achenes, it is seen to match very closely the Bolivian specimens of var. *decomposita*, except in the unimportant respect that it has somewhat larger rays.

[4] Unfortunately, *B. triplinervia* produces at times an 8-rayed form. Discussion of this form must be deferred until a later date.

[5] To be treated in a subsequent paper.

Bidens urophylla, sp. nov. (Pl. II)—Herba glabra, verisimiliter perennis scandensque, forsitan 1–3 m. alta vel longa; ramis aegre angulatis vel teretibus. Folia petiolata petiolis tenuibus 2.5–4.5 cm. longis, petiolo adjecto 8–14 cm. longa, pinnatim 3–5-partita, foliolis anguste lanceolatis, membranaceis, usque ad 1.6 cm. latis, valde et perspicue (prominentibus anguste linearibus 1–3.5 cm. longis) caudato-acuminatis, pauciserrata unico latere 2–6 mucronatis dentibus munito, margine eciliatis. Capitula radiata, pansa ad anthesin ±3 cm. lata et 0.9–1.4 cm. alta, maximam partem verisimiliter corymboso-paniculata. Involucri bracteae exteriores 6–8, anguste lineares, subglabrae, crassiusculae, apice subacutae, 5–8 mm. longae; interiores latiores et paulo longiores. Flores ligulati 5 vel 6, sicci albo-flavidi, ligula oblongo-elliptici, apice denticulati, ±1.5 cm. longi. Achaenia matura non visa; sub matura fusco-nigra, linearia, plana vel subtetragona, corpore 9–12 mm. longa et circ. 1 mm. lata, omnino etiam ad margines glabra vel ad summam sparsissime setulosa, biaristata aristis supra retrorsum infra antrorsum hamosis, tantum circ. 1 mm. longis.

Karl Friedrich Philipp von Martius, in thickets ("sepibus") at the margins of forests near Mariana, Minas Geraes, Brazil, in years 1817–1820 (April; type in Herb. Univ. Munich).

The type had been labeled *Bidens rubifolia* H.B.K., probably by J. G. Baker. Indeed, from its general habit, the species does appear to be without doubt a close relative of such species as *B. rubifolia* H.B.K., *B. squarrosa* H.B.K., *B. speciosa* Gardn., *B. reptans* (L.) G. Don, *B. Holwayi* Sherff, and *B. Shrevei* Britton, all of them perennial climbers. From these it differs decidedly, however, in its remarkably caudate-tipped leaflets and in its achenes, which are almost entirely glabrous, even on the margins, and have only short aristae.

Bidens australis Spreng. Syst. **3**:453. 1826; *Coreopsis fruticosa* Forst. Prodr. Fl. Ins. Austr. 91. 1786 (*nomen; non* Vest); *Campylotheca australis* (Spreng.) Less., Linnaea **6**:509. 1831; *Bidens paniculata* Hook. and Arn. Bot. Beech. Voy. 66. 1841; *B. fruticosa* Schz. Bip., Flora **39**:358. 1856 (*non* L., *non* DC.); *Coreopsis fruticosa* Solander *mss.*, in Seeman Fl. Vitiensis 143. 1865–1868.

In 1769, Banks and Solander collected fine material of this species on the Society Islands (Tahiti, *fide* Sol. in Seem. *l.c.*) and on the Friendly Islands. These collectors were attached to Capt. Cook on the first of his three famous voyages (*cf.* Encycl. Brit. edit. 11, **3**:333. 1910). Further specimens were col-

lected at Tahiti on Capt. COOK's third voyage. Both sets of material are still extant in excellent condition (Herb. Brit. Mus.). A sheet from the first voyage (Society Islands) bears the determination *Coreopsis fruticosa* Mscr.[6] One from the third voyage bears the name *Coreopsis fruticosa* Sol. In SOLANDER's unpublished manuscript, at the British Museum of Natural History, is his very complete and precise description of these plants under the name *Coreopsis fruticosa*. This description was not published until 1865–68 (SEEMANN, *l.c.*).

Meanwhile, FORSTER, who was botanist on Capt. COOK's third voyage (*cf.* Encycl. Brit. edit. 11, 10:674. 1910), listed a *Coreopsis fruticosa* with the habitat "*intra tropicos*." He gave no description, and so the name, with him, amounts to merely a *nomen nudum*. In fact, it seems entirely plausible that he meant merely to list a plant collected by him on COOK's third voyage and which he had found to match SOLANDER's contemplated species that had been collected on COOK's first voyage. FORSTER's small and rather scanty private specimen went into the hands of SPRENGEL, who gave the first published description of it under the new name *Bidens australis*. This historic fragment later became the possession of SCHULTZ BIPONTINUS, still later of E. COSSON, and now is in Paris (Herb. Mus. Hist. Nat.). It agrees precisely with the material in London, already mentioned as having been collected likewise on COOK's third voyage. SCHULTZ BIPONTINUS (*l.c.*) gave a very full description of FORSTER's fragment, evidently unaware of the much more ample duplicate material in London. Nor does he seem to have known of the synonymous *Bidens paniculata* Hook & Arn., based on Capt. BEECHEY's plant from Tahiti (type in Herb. Kew).

The five fruiting heads remaining on FORSTER's private fragment have achenes measuring, aristae included, about 3 mm. long (*cf.* Schz. Bip. *l.c.*, "1 Linie lang, oder etwas länger"). Those on the duplicate material studied by SOLANDER vary in length from 3 to 6 mm. (*cf.* Sol. in Seem. *l.c.*, "bilinearia"). Those on the type of *B. paniculata* Hook. & Arn. average slightly smaller, varying from 2.5 to 3.9 mm. long, but this difference seems without much significance.

ASA GRAY (Proc. Amer. Acad. 5:128. 1861) erroneously associated this species with the Hawaiian *B. sandvicensis* Less. DRAKE (del Castillo, Ill. Fl. Ins. Mar. Pacif. 209–210. 1890) erroneously referred it to *Bidens Menziesii* (Gray) Sherff (*Coreopsis Menziesii* Gray), but he had already given a good picture of it under the name *Bidens paniculata* H. & A. (*l.c.* Pl. 40. 1888). From its general habit, also its much smaller and more numerous heads, it is seen to be affiliated more closely with such species as *B. polycephala* Schz. Bip. and *B. Ahnnei* Sherff, both of the southern Pacific, than with species such as *B. sandvicensis* Less. of the Hawaiian region. Nor does *B. australis* resemble at all closely *B. lantanoides* Gray, which SEEMANN (*l.c.*) thought was "probably identi-

[6] The BANKS and SOLANDER specimen at Paris is from the Friendly Islands (1769). It matches the Society Island plants, but the label bears none of SOLANDER's own notations.

cal." From the specimens studied by me, SPRENGEL's meager description may now be amplified as follows:

BIDENS AUSTRALIS Spreng., descript. amplific.—Suffruticosa, glabra, usque ad 1.8 m. alta; caule obtuse tetragono vel subtereti, erecto, ramoso vel saepe ramosissimo. Folia tenuiter petiolata petiolis 1–3 cm. longis, petiolo adjecto 6–13 cm. longa, indivisa, lanceolata oblongave et apice plerumque acuminata, serrata dentibus parvis et plerumque 20–40 in unico latere, non ciliata. Capitula parva, paniculato-corymbosa, supra folia exserta, minute radiata, ad anthesin 6–7.5 mm. lata et 3–4 mm. alta, tenuissime pedunculata pedunculis 1–3 cm. longis. Involucrum vix hispidulum vel profecto saepe glabrum; bracteis exterioribus 5–7, minimis, linearibus, supra saepe dilatatis, apice plerumque subobtusis, raro subciliatis, circ. 1 mm. longis; interioribus lanceolatis, circ. 2 mm. longis. Flores ligulati circ. 5, minimi, ligula late ovati vel oblongi, apice plerumque denticulati, flavi, 2–3 mm. longi. Achaenia minuta, linearia, infra parce angustata, obcompresso-tetragona vel subplana, atra, sparsim suberecto-setosa, paleas saepe superantia, corpore 2.5–6.6 mm., brevissime biaristata aristis divergentibus, retrorsum hamosis, 0.2–0.5 mm. longis.

Specimens examined.—*Banks* and *Solander*, Tahiti, 1769 (Herb. Brit. Mus.); *iidem*, Friendly Islands (Herb. Paris); Capt. *Beechey*, Tahiti (Herb. Kew); *Bertero* and *Moerenhout*, Tahiti, 1831 (Herb. E. Drake in Herb. Paris); *Bidwill*, Morea (Eimeo; Herb. Kew); *J. G. Forster*, Tahiti (Herb. Brit. Mus.; Herb. Paris); *Lepine* 98, Tahiti (Herb. Kew); *Moerenhout*, Tahiti, 1834 (Herb. Paris); *J. Nadeaud* 336, on precipices, Tahiti, June 1, 1857 (Herb. E. Drake in Herb. Paris); *Webb*, Tahiti (Herb. Kew).[7]

Bidens Mathewsii, sp. nov.—Glabra, supra herbacea, infra verisimiliter suffruticosa, forsitan 6–10 dm. alta, ramis angulatis. Folia tenuiter petiolata petiolis 1–2 cm. longis, petiolo adjecto 7–10 cm. longa, indivisa, lanceolata vel oblongo-lanceolata, membranacea, serrata dentibus parce mucronatis, eciliata, apice acuta. Capitula pauca (±8 in unico ramo), corymbosa, supra folia exserta, radiata, pansa ad anthesin ±0.8 cm. alta et ±1.3 cm. lata. Involucri brac-

[7] CHAMISSO's specimens from Oahu Island, cited by LESSING (Linnaea 6:509. 1831) for this species, are merely fragments with branchlets and leaves, but more or less deficient as to heads (Herb. Univ. Halle, Herb. Kew). They match *B. micrantha* Gaud., a Hawaiian species with compound leaves and very different from *B. australis*.

teae exteriores 8–10, oblongo-lineares, glabratae, apice obtusae, 3–6 mm. longae; interiores lanceolatae, paulo longiores, versus basim saepe minute pulverulentae. Flores ligulati ±5, lutei, ligula elliptico-oblongi, apice plus minusve denticulati, ±7 mm. longi. Achaenia paleis elongatis parce aequantia, plerumque anguste rarius late linearia, plana vel obcompresso-triquetra, exalata, atra, dense erecto-hispida pilis stramineis, corpore 5–8 mm. longa et 0.5–1 mm. lata, apice biaristata aristis retrorsum hamosis 0.5–1 mm. longis.

A. Mathews 110, Pitcairn Island, March, 1830 (type in Herb. Kew).

A plant with the foliage habit of *Bidens australis* Spreng., but having fewer and larger heads, more numerous and more uniformly linear (not apically dilated) exterior involucral bracts, and larger, much more hispid achenes.

BIDENS PILOSA, var. ALAUSENSIS (H.B.K.), comb. nov.; *B. alausensis* H.B.K. Nov. Gen. et Sp. 4:184 (235). 1820; *B. valparadisiaca* Colla, Mem. Accad. Torin. 38:12. *pl. 24.* 1835; *B. chilensis* DC. Prodr. 5:603. 1836; *B. valparadisea* Colla *ex* Philippi Cat. Pl. Chil. 155. 1881.

In a former article I have discussed the identity of *B. valparadisiaca* Colla and *B. chilensis* DC. with *B. alausensis* H.B.K., and presented photographs of KUNTH's type of *B. alausensis* H.B.K. (BOT. GAZ. 59:310–311. *fig. 1.* 1915). In 1924 I made a careful reexamination of KUNTH's type and four sheets of cotype material (Herb. Paris, Herb. Berl.) and came again to the same conclusion as before. A study of various other specimens, however, shows that *B. alausensis* H.B.K. cannot properly be regarded as specifically distinct from *B. pilosa* L. Thus, for example, *J. Francis Macbride* 2899 and 2901, at altitude of about 8000 ft., Matucana, Peru, March 14–18, 1923 (Herb. Field Mus.)[8] and *Poeppig* 207, *prope* Valparaiso, Chile (type and cotypes of *B. chilensis* var. *apiifolia* DC.; Herb. DC., Herb. Mus. Vienna, Herb. Univ. Kiel, etc.) have tripartite leaves and are forms of *B. pilosa* var. *radiata* Schz. Bip.[9] But *Macbride* 2902 (Herb. Field Mus.), collected at the same place and same time as *Macbride* 2899 and 2901, has the foliage mostly bipinnatisect, and is very close to or identical with various specimens of *B. alausensis* H.B.K. from Chile and Ecuador. *Macbride* 2900, altitude about 8000 ft., Matucana, Peru, March 14–18, 1923 (Herb. Field Mus.), and 3473, at altitude of about 8500 ft., Cani, Peru, April

[8] These and the other MACBRIDE specimens cited were very kindly obtained for me by Mr. MACBRIDE, who had the matter particularly in mind on his recent South American Expedition. Of each number cited I have seen all the duplicates before their distribution to other herbaria.

[9] Having rays mostly 5–8 mm. long and slightly more yellowish than usual in the var. *radiata*. They suggest in the ray characters the allied forms that pass under the name *B. pilosa* var. *minor* (Bl.) Sherff.

16–26, 1923, also are identical in habit and technical characters with *B. alausensis*, except that they are taller and more robust and their foliage is more finely cut, becoming even tripinnatisect. These are identical with *A. D'Orbigny* 1234, Bolivia (Herb. Paris), labeled *Bidens scandicina* var. *glabrescens* by WEDDELL. They match also the Berlin and Paris cotypes (now before me) of *B. scandicina* H.B.K., except that the latter have more pubescent leaves with somewhat finer divisions. It is significant that the types of *B. alausensis* H.B.K. and *B. scandicina* H.B.K., here seen to be so closely connected by intermediate specimens, were collected by HUMBOLDT and BONPLAND in the same vicinity: *B. alausensis* between Alausi and Tambo de Guamote, Ecuador, and *B. scandicina* between Llactacunga and Hambato, Ecuador.

It might seem at first that we have here a situation comparable with that in *B. pilosa* var. *bimucronata* (Turcz.) O.E.S., where the form with finely divided foliage is reducible to subordinate rank as f. *odorata* (Cav.) Sherff. In such a case we should have the names *alausensis* and *scandicina* taking respectively *formal* and *subformal* rank under the name *B. pilosa* var. *radiata*, giving names which would be very cumbersome. It seems more probable, however, that in the present case the *alausensis* and *scandicina* forms do not intergrade nearly so much with *B. pilosa* var. *radiata* as does the *odorata* form with *B. pilosa* var. *bimucronata*. Hence there appears good reason for assigning *B. alausensis* varietal status directly under *B. pilosa*, and reducing *B. scandicina* to a *forma* under the variety:

B. PILOSA var. ALAUSENSIS f. SCANDICINA (H.B.K.), comb. nov.

Specimens examined, (*a*) var. *alausensis*.—*J. Ball*, alt. 12,000–13,000 ft., *ex saxosis andium peruviae juxta pagum* Chicla, April 21–23, 1882 (Herb. Kew); *D. Bertero* 845 *pro parte*, Quillota, Chile, October–November, 1829 (type of *B. chilensis* DC. in Herb. DC.; cotype, Herb. Par.); *idem* 845 *pro parte, in pascuis sylvaticis et ad ripas collium*, Valparaiso, Chile, 1830 (Herb. Mus. Vienna; Herb. N.Y. Bot. Gard.; Herb. Brit. Mus.; Herb. Mun.); *Bridges*, Valparaiso, Chile, 1830 (Herb. Kew); *idem* 66, Valparaiso, Chile, 1832 (Herb. Brit. Mus.; Herb. Kew; Herb. Mus. Vienna); *Otto Buchtien*, Valparaiso, Chile, August 20, 1895 (Herb. Field Mus.); *Agnes Calvert*, Valdivia and Valparaiso, Chile (Herb. Brit. Mus.); *Cruckshank*, Chile (Herb. Kew); *H. Cuming* 646, *prope* Valparaiso, Chile 1831 (Herb. Brit. Mus.; Herb. Mus. Vienna; Herb. Webb); *Frömbling*, Chile, 1886 (Herb. Mun., 2 sheets); *Gaudichaud* 164, Valparaiso, Chile, 1834 (Herb. DC.; Herb. Webb.); *Cl. Gay* 201, Chile, 1833 (Herb. DC.; Herb. Gray, 2 sheets); *idem* 341, *in collibus*, Prov. de Coquimbo, Chile, August, 1829 (Herb. Par.); *idem* 355, *in collibus*, Chile (Herb. Par.); *W. H. Harvey*, Valparaiso, Chile, April–July, 1856 (Herb. Gray); *Humboldt* and *Bonpland*, alt. 7800 ft., between Alausi and Tambo de Guamote, Ecuador (type and cotype in Herb. Par.); *J. Francis Macbride* 2902, on slide rock, alt. about 8000 ft., Matucana, Peru, March 14–18, 1923 (Herb. Field Mus.); *Macrae*, *prope* Valparaiso, Chile, 1825 (Herb. Kew); *Mathews* 465, in interior Peru, 1862 (Herb. N.Y. Bot. Gard.); *idem* 468, Pur-

ruchuco, Peru (Herb. Kew); *Herb. Miers* 67, Concon, Chile (Herb. Brit. Mus.); *Mosely* (Challenger Exped.), Juan Fernandez, Chile, November, 1875 (Herb. Kew); *F. W. Neger*, Concepcion, Chile, July 20, 1895 (Herb. Mun.); *F. Philippi*, Valparaiso, Chile, 1894 (Herb. Brit. Mus.); Mr. and Mrs. *J. N. Rose* 19114, near Valparaiso, Chile, September 14, 1914 (Herb. U.S. Nat.); *R. E. Snodgrass* and *E. Heller* 887, at alt. 4000 ft., Tagus Cove, Albermarle Island, Galapagos Islands, June 15, 1899 (Herb. Gray); *Alban Stewart* 716, abundant in thickets at alt. 4000 ft., Tagus Cove, Albermarle Island, Galapagos Islands, March 24, 1906 (Herb. Brit. Mus., Herb. Mo. Bot. Gard.); Dr. *Wawra* (H. M. Frigate "Donau"), Valparaiso, Chile, 1868–1871 (Herb. Mus. Vienna).

Specimens examined, (*b*) f. *scandicina*.—*Otto Buchtien*, alt. 3500 m., La Paz, Bolivia, May, 1911 (Herb. Mun.); *idem* 378, at alt. 3460 m., La Paz, Bolivia, March 8, 1919 (Herb. Field Mus., 2 sheets); *idem* 811, at alt. 3460 m., La Paz, Bolivia, March 8, 1919 (Herb. Field Mus., 2 sheets); *idem* 811, at alt. 3460 m., La Paz, Bolivia, March 8, 1919 (Herb. Field Mus., 2 sheets).[10] *Const. de Jelski* (*distrib. Dr. Ign. de Szyszylowicz*) 657 and 735, Cutervo, Peru, April, 1879 (Herb. Berl.); *A. D'Orbigny* 1234, Bolivia (Herb. Par., *sub. nom. B. scandicina* var. *glabrescens* Weddell); *Humboldt* and *Bonpland*, between Llatacunga and Hambato, Ecuador (type and 2 cotype sheets in Herb. Par.; 2 cotype sheets in Herb. Berl.); *J. Francis Macbride* 2900 *pro parte*, very rocky valley floor, alt. 8000 ft., Matucana, Peru, March 14–18, 1923 (Herb. Field Mus., where two of the duplicate sheets bear plants with less divided foliage and are referable to the var. *alausensis* proper); *idem* 3473, alt. about 8500 ft., Cani (pueblo 7 miles northeast of Mito), Peru, April 16–26, 1923 (Herb. Field Mus.); *A. Sodiro, in campis interandinis prope* Pomasqui, Ecuador, February, 1896 (Herb. Berl., 2 sheets).

BIDENS FERULAEFOLIA (Jacq.) DC. Prodr. 5:603. 1836; *Coreopsis ferulaefolia* Jacq. Hort. Schoenbr. 3:65. *pl. 375.* 1798; *Bidens procera* D. Don, Bot. Reg. 8:684. *pl. 684.* 1822; *Coreopsis angustifolia* Pavon *ex* D. DON *l.c.; Kerneria ferulaefolia* (Jacq.) Cass. Dict. 51:473. 1827.

The type of *B. procera* Don had been raised from seed sent by DON JOSE PAVON to Mr. LAMBERT. PAVON had received the material from Mexico, but the exact locality in Mexico is not stated by DON. I have not knowingly seen the Lambert Herbarium specimens which DON cited, but there lies before me an

[10] In the BUCHTIEN specimens the similarity to certain Umbelliferae, implied in the name *scandicina*, is most striking. In fact, before seeing the SODIRO (*cf.* HIERONYMUS, Engler Bot. Jahrb. 29:48. 1901) and DE JELSKI specimens, I myself had not thought to connect the BUCHTIEN specimens with f. *scandicina*, and had tentatively considered them as representing a new species. Curiously enough, I too had been impressed with the resemblance to certain Umbelliferae, especially *Musineon*, and, to a lesser extent, *Chaerophyllum*, and had tentatively employed a trivial name based upon this resemblance. In one specimen by BUCHTIEN (no. 811) the foliage and general habit suggest also those of *Anthemis Cotula* L.

original specimen of PAVON'S own herbarium (*ex* Herb. Boiss.). This agrees with DON'S description and plate fairly well, the chief differences being attributable to the fact that DON based his description mainly on the taller and more robust cultivated plants. Thus, early in his description, DON described his plant as "*orgyalis v. ultra*," and later on he described it as "6 or 8 ft. high." I have seen no spontaneous specimens that measured nearly so tall as in DON'S cultivated plants. These latter, however, are seen from DON'S description and plate to match the original plate and the various cultivated specimens (extant in European herbaria) of *B. ferulaefolia* (Jacq.) DC.

Most of the spontaneous specimens have a low stature (5–8 dm. high) and slender branches; very rarely have they been found to resemble the cultivated plants (illustrated especially well in JACQUIN'S plate), and then they appear to have grown in an aqueous habitat.

Specimens examined.—*Frère G. Arsène* 3080, vicinity of Morelia, Michoacan, Mexico, in 1910 (Herb. Berl.); *E. Bourgeau* 502 *pro parte* Guadalupe (Hidalgo), near City of Mexico, Mexico, August 23, 1865–66 (Herb. Berl.; Herb. Boiss.; Herb. U.S. Nat.); *C. V. Hartman* 47, in water ditch, Fronteras, Sonora, Mexico, September 25, 1890 (Herb. Gray); *idem* 834, San Pedro, Sonora, Mexico, September 14, 1890 (Herb. Gray); *idem* 961, between San Pedro and Fronteras, Sonora, Mexico, September 20–24, 1890 (Herb. Gray); *idem* 991, Fronteras, Sonora, Mexico, September 25–29, 1890 (Herb. Gray); *Herb. Fauche* 50 (Herb. Boiss.); *J. Gregg* 397, Mexico, 1848–49 (Herb. Gray); *Jacquin* fil., cult. in 1809 (Herb. DC. Prodr.); *J. G. Lemmon*, southern Arizona, 1881 (Herb. Gray); Mr. and Mrs. *J. G. Lemmon*, Rucker Valley, Chiricahua Mts., Arizona, September, 1881 (Herb. Brit. Mus.; Herb. Univ. Calif.); *iidem* 2768, spring at "Hermitage," Rucker Valley, Chiricahua Mts., Arizona, September, 1881 (Herb. Univ. Calif.); *Hort. Bot. Monac.*, in 1845 (Herb. Mun., 4 sheets); *Edward Palmer* 316 and 393, southwestern Chihuahua, Mexico, August to November, 1885 (Herb. Gray); *idem* 425 and 426, Guadalajara, Jalisco, Mexico, July to October, 1886 (425, Herb. Univ. Vienna; 426, Herb. Boiss.; Herb. U.S. Nat.); *idem* 668, 672, and 677, vicinity of City of Durango, Durango, Mexico, April to November, 1896 (668, Herb. Univ. Calif.; 672, Herb. U.S. Nat.; 677, Herb. Berl. and Herb. Gray); *idem* 933, wet bottoms along water courses, vicinity of City of Durango, Durango, Mexico, July to November, 1896 (Herb. Berl.; Herb. Boiss.; Herb. Gray; Herb. Univ. Calif.); *Hort. Paris.*, November 1, 1814 (Herb. Kew); Herb. PAVON, *sine patria et sub nom. Coreopsis tripinnatifida* (Herb. Boiss.); *idem*, Mexico (Herb. Boiss.); Hort. *Pelon*, November, 1819 (Herb. Kew); *C. G. Pringle* 136, Mexico (Herb. Gray); *J. N. Rose* 2530, Huejuquilla, Jalisco, Mexico, August 24, 1897 (Herb. Gray); *J. T. Rothrock* 671, Sannoita Valley, southern Arizona, September, 1874 (Herb. Gray); *Schaffner*, near St. Angelum, Mexico, September, 1855 (Herb. N.Y. Bot. Gard.); *idem* 213, Valley of Mexico, Mexico (Herb. Berl.; Herb. Gray); *W. Schumann* 108, river bank, Faral, Mexico, September 10, 1885 (Herb. Berl. 2 sheets; Herb. Univ. Vienna); *George Thurber* 1102, Sonora, Mexico, September 11, 1851 (Herb. Field Mus.; Herb. Gray);

J. W. Toumey 58, Chiricahua Mts., Arizona, July 26, 1899 (Herb. Univ. Calif.); *C. H. T. Townsend* and *C. M. Barber* 315, altitude of 7200 feet, Chihuahua, Mexico, September 6, 1899 (Herb. Boiss.); *C. Wright* 1232, Sonora, Mexico, in 1851 (Herb. Gray); *Herb. Zucarini*, cult. in 1817 (Herb. Mun.).

BIDENS FERULAEFOLIA var. FOENICULAEFOLIA (DC.), comb. nov.; *B. foeniculaefolia* DC. Prodr. **5**:603. 1836; *Coreopsis foeniculacea* Moc. and Sesse *ex* DC. *l.c.*

The type material of *B. foeniculaefolia* DC. was collected in 1829, partly by MENDEZ and partly by ALAMAN, some plants to the west and some to the south of Guanajuato, Leon, Mexico. Besides the four type sheets in the De Candolle Prodromus Herbarium, duplicates are in Paris and Florence. ASA GRAY suspected *B. foeniculaefolia* DC. of belonging to *B. procera* (Synopt. Fl. 1^{II}:298. 1884; *cf.* Proc. Amer. Acad. **19**:16. 1884). The resemblance is very close, but *B. foeniculaefolia* tends to have a more wiry aspect, with narrower leaf divisions, and more pubescence. The achenes are less numerous and less compactly arranged in the heads than those of typical *B. procera*, or *B. ferulaefolia* as we must call it. Furthermore, their bodies average about 8 mm. long and 0.6 mm. wide, while the bodies of *B. ferulaefolia* are usually shorter and wider than this. The aristae are usually 2, but at times 3 or 4,[11] while in *B. ferulaefolia* they seem constantly 2. At the most, however, *B. foeniculaefolia* does not exhibit enough differences from *B. ferulaefolia* to warrant more than varietal distinction. Besides the original specimens, I have examined the following: *J. Gregg* 397, Mexico, September 1, 1848–49 (Herb. Mo. Bot. Gard.); *Nicholas*, Puebla, Mexico, June 9, 1910 (Herb. Par.); *Edw. Palmer* 681, vicinity of City of Durango, Durango, Mexico, September, 1896 (Herb. Berl.); *George Thurber* 1102, Sonora, Mexico, September, 1851 (Herb. Field Mus.).

BIDENS FERULAEFOLIA var. LUDENS (A. Gray), comb. nov.; *B. ludens* A. Gray, Proc. Amer. Acad. **21**:390. 1886.

ASA GRAY, shortly before his death, applied the name *B. ludens* to a plant collected by PRINGLE, no. 293, hills and plains, northwest of the City of Chihuahua, Chihuahua, Mexico, October, 1885 (Herb. Gray). GRAY's type specimens do not differ much in general appearance from *B. procera*. The fruiting heads, however, are noticeable because most of the achenes lack or have dropped their aristae.[12] In certain of the foregoing collections cited for *B. ferulaefolia* proper, however, particularly some of PALMER's plants, there are heads in which most of the achenes have likewise lost their aristae. Indeed, DON himself had noted this as one of the characters of his *B. procera*.[13] Another character noted in

[11] DE CANDOLLE is seen, from his description ("biaristatis"), to have overlooked the additional aristae on his own material.

[12] ". . . . aristis subulatis . . . . (persistentibus vel deciduis)"—GRAY, *l.c.*

[13] ". . . . aristis . . . . deciduis . . . . having flat cuneiform seed with deciduous (not permanent) awns." DON, *l.c.*

GRAY'S type material is the usually greater proportionate length of the mature paleae. In most of the heads they are larger than the achenes, but on the same plant some heads may be found with exceptionally long achenes, these surpassing the paleae. Evidently GRAY'S *B. ludens* is best regarded as merely a variety of *B. ferulaefolia*. Besides the type material, I have examined the following specimens: *E. W. Nelson* 1438, altitude of 5500–7500 feet, Valley of Oaxaca, Oaxaca, Mexico, September 20, 1894 (Herb. Gray; Herb. U.S. Nat.); *C. G. Pringle* 757 *pro parte*, mountains near City of Chihuahua, Chihuahua, Mexico, October 3, 1886 (Herb. Berl.; Herb. Brit. Mus.; Herb. Univ. Vienna).

BIDENS ABADIAE DC. **pilosoides,** var. nov.—A specie foliis pinnatim 3–5-partitis foliolis ovatis vel terminali lanceolato omnibus serratis vel incisis apice obtusis vel acutis capitulis plerumque eradiatis diversa; habitu *B. pilosae* adpropinquans.

H. Cuming 1041, Lima, Peru, in 1831 (type and cotype in Herb. Kew); *W. Nation*, cultivated places, Lima, Peru, in 1862 (Herb. Kew); ex Herb. Pavon, Peru (Herb. Webb).

Bidens Abadiae DC. was founded upon a specimen collected at Lima, Peru, in 1833 (Herb. DC.). The leaves of the type are bipinnatisect, and the general aspect of the foliage is comparable with that for *B. pilosa* var. *bimucronata* f. *odorata* (Cav.) Sherff. In the Boissier Herbarium is a plant from the Pavon Herbarium and collected at Lima or vicinity, which matches the type very closely in its bipinnatisect foliage. The technical characters of both the old and young heads on these plants are very similar to those often met with in *B. pilosa* L. The general habit is so distinct, however, that to equate *B. Abadiae* and *B. pilosa* would seem unjustifiable.

In the Webb Herbarium at Florence is still another specimen from the Pavon Herbarium, collected in Peru. This has the *leaves merely tripartite*, with the terminal lobe subtripartite. The specimens by CUMING and by NATION, all from Lima, likewise differ from the DE CANDOLLE type in having *leaves only once divided*. One of the CUMING specimens has leaves approaching those of the related *B. pilosa* var. *alausensis* (H.B.K.) Sherff. As the difference between the simply pinnate and the bipinnatisect types of foliage appears here to be emphatic, as in the varieties of *B. pilosa*, I have given the name *pilosoides* to the variety having 3–5-partite leaves.

BIDENS PILOSA var. BIMUCRONATA (Turcz.) O. E. Schulz, Urban Symb. Antill. 7:138. 1911; *B. bimucronata* Turcz., Bull. Soc. Nat. Mosc. 24:184. 1851; *B. caracasana* DC. Prodr. 5:600. 1836.

The name *Bidens caracasana* DC. has been carelessly treated by botanists, having been referred to a number of widely different species. The De Candolle Prodromus Herbarium (Herb. Deless.) contains two sheets of type material,

Vargas 210, Caracas, Venezuela, in 1830. All of this material is the form understood by me (*ex descript. et patr.*) to represent *B. bimucronata* Turcz., recently reduced by O. E. SCHULZ (*l.c.*) to a variety of *B. pilosa* L.

BIDENS PILOSA var. BIMUCRONATA forma ODORATA (Cav.), comb. nov.; *B. odorata* Cav. Icon. 1:9. *pl. 13.* 1791; *Coreopsis ferulaefolia* var. *odoratissima* Pers. Synops. Pl. 2:477. 1807; *C. multifida* DC. Prodr. 5:573. 1836; *C. multifida* var. *mutica* DC., *l.c.; B. daucifolia* DC., *l.c.* 601; *Bidens ferulaefolia* var. *odoratissima* (Pers.) DC., *l.c.* 603. 1836; *B. caucalidea* DC., *l.c.* 604; *B. Deamii* Sherff, BOT. GAZ. 56:490. 1913; *B. ramosissima* Sherff, *l.c.* 491.

For a discussion of *Bidens odorata* Cav. and of *Coreopsis ferulaefolia* var. *odoratissima* Pers., reference is made to an earlier paper (BOT. GAZ. 59:304. 1915).

Coreopsis multifida DC. and its var. *mutica* DC. are represented by the type specimens in the Herbarium of De Candolle's Prodromus (Herb. Deless.). The species was collected (*ex* DC., *l.c.*) by PAVON, possibly in Peru; the variety was from seed previously sent by DOMBEY from Peru to the Botanical Garden at Paris. Both are found on careful comparisons to be merely *Bidens odorata* Cav.

Bidens caucalidea DC. was described from *Berlandier's* 1138. The sheet of this number in the Museum of Vienna has one specimen with bipinnate leaves, approaching *B. odorata* Cav., and another with some of the principal leaves only tripartite, approaching *B. pilosa* var. *bimucronata* (Turcz.) O. E. Schulz. At Washington (U.S. Nat. Herb.) are two sheets of the fine material collected by ARSÈNE. It is remarkably close to the type material of *B. caucalidea* and might well serve as a supplementary type for the concept represented by that name. Four of the five specimens there have the leaves mainly bipinnate as in *B. odorata* Cav., but the remaining specimen has the leaves tripartite as in *B. pilosa* var. *bimucronata.*[14] Obviously the amount of foliar division is of little value here in determining specific limits. Likewise, a study of various other specimens (referred to *B. odorata* Cav. or to *B. caucalidea* DC.) with reference to achenial shape, size, and armature, to width of flowering heads and to general branching habit, reveals an astonishing amount of variation. A form with the upper part of the main stem broken away and the lower part profusely branched is the plant formerly described as *Bidens Deamii* Sherff. *B. ramosissima* Sherff is probably best interpreted as merely a mutant form with an excessive degree of branching, and the involucres finally becoming reflexed to a notably uniform extent. A specimen collected by SEEMANN (Herb. Kew) is positively the *B. odorata* of CAVANILLES, and yet in its highly branched character displays an approach to the type of *B. ramosissima* (U.S. Nat. Herb.).

[14] *Cf.* O. E. SCHULZ, Urban Symb. Antill. 7:138. 1911; "Bidens caucalideus DC.! . . . . varietati *bimucronato* valde affinis folia pinnatisecta habet."

Certain specimens of *Berlandier's* 1138 were rather under-developed in stature, somewhat pubescent-hirtellous, and had the leaf segments more acute. These DE CANDOLLE assumed to be another species, confused with the *B. caucalidea* material by BERLANDIER in the gathering. He named them *B. daucifolia.* For *B. daucifolia*, as for his *B. caucalidea*, he described the rays as yellow. Since DE CANDOLLE's day, many yellow-rayed Mexican specimens related to *B. andicola* H.B.K. have been determined by students (myself among them) as *B. daucifolia* DC.;[15] but my recent study of the long neglected type material of *B. daucifolia* at Geneva (Herb. DC.) and Paris (Herb. Mus. Hist. Nat.) showed that *B. daucifolia* is not a yellow-rayed form. The Geneva sheet has a single small specimen, with one flowering and one fruiting head. The ligules are a faded rosaceous color, not yellow as DE CANDOLLE had stated. The achenes are those of *B. odorata* Cav. The Paris sheet has three small but good specimens. Three radiate heads are present, of a faded rosaceous color. One specimen has a head of biaristate achenes and another has two ample fruiting heads of exaristate achenes. These specimens all are mere variations of *B. caucalidea* DC., and there is no reason to suppose that BERLANDIER confused two sorts with each other. Clearly then, *B. daucifolia* DC. and *B. caucalidea* DC. are synonymous, and together must be equated in turn with the earlier *B. odorata* Cav.

A study, in herbaria, of the numerous specimens referable to *B. odorata* Cav., shows no less than eight or ten *facies* or aspects to be found. These might seem to represent a corresponding number of varieties and racial forms. To encumber taxonomy with a great number of additional names, however, when the variations are so capricious and fickle as often to result in two or three unlike *facies* upon a single herbarium sheet, would appear entirely unwarranted. These varying forms, therefore, all of them intimately related to *B. pilosa* var. *bimucronata* but tending to display more or less bipinnate leaves on most of the individual plants, are grouped together by me as the forma *odorata* of that variety.[16] Complicated and undesirable as the name resulting from this interpretation will be, nevertheless it is the only one that appears to accord with the facts in nature.

BIDENS AUREA (Ait.[17]) Sherff, BOT. GAZ. 59:313. 1915 (*ex synon.* Ait. *nec alior*); *Coreopsis aurea* Ait., Hort. Kew 3:252. 1789 (*non auctores*); *Bidens heterophylla* Ortega Hort. Matr. 99. *pl.* 12. 1798;

[15] Already mentioned under *B. andicola.*

[16] O. KUNTZE (Rev. Gen 1:322. 1891) put forth the even more complicated and unwelcome method of proposing varieties, subvarieties, and forms (*formae*). His name *subbiternata* is seen, on carefully reading the related names, to have been used for a subvariety or group (under *B. pilosa*) ranking lower than a variety but higher than a *forma* (thus *cf. B. pilosa* α *leucantha* 2. *ternata* f. *pilosior*, *l.c.*). Even were the *formal* rank of the name *subbiternata* accepted, however, it could not conflict with the validity of the claim of *odorata* to *formal* rank, as *subbiternata* pertained to a form of *B. pilosa* var. *radiata* Schz. Bip., not var. *mucronata* (Turcz.) O. E. Schz.

[17] Less commonly, but probably with greater justice, cited "Dryander in Aiton."

B. luxurians Willd. Enum. Hort. Berol. 847. 1809 (*nec alior*); *B. arguta* H.B.K. Nov. Gen. et Sp. **4**:181 (231). 1820; *B. decolorata* H.B.K. *l.c.* 182 (233); *B. arguta* var. *luxurians* DC. Prodr. **5**:596. 1836; *B. longifolia* DC. *l.c.* 597; *B. Warszewicziana* Regel *cum vars.* α, β, γ. Flora **32**:183–184. 1849; *B. heterophylla* var. *Wrightii* Gray, Proc. Amer. Acad. **19**:15. 1883; Synopt. Fl. N. Amer. **1**$^{\text{II}}$:298. 1884; *B. heterophylla* var. *typica* Fiori *in* Fiori e Paoletti Fl. Anal. Ital. **3**:303. 1904.

The name *Coreopsis aurea* Ait. has been associated by botanists for more than a century with a plant native to the southeastern United States. The description given by AITON is rather short: "aurea 2 *Coreopsis* foliis serratis: radicalibus tripartitis; caulinis trifidis integrisve lanceolato-linearibus. Hemp-leav'd *Coreopsis*. Nat. of North America. Introd. 1785 by CHARLES EARL of Tankerville. Fl. August & September. H. ♃." The specimen collected from the type plants in Kew Garden in 1785, however, is still extant in good condition in the British Museum of Natural History.[18] This specimen bears a superficial resemblance to the plant of the southeastern United States, *Coreopsis mitis* Michx., and from the confusion found in literature, appears to have deceived every botanist who had examined it. I myself misinterpreted it in 1914 (BOT. GAZ. **59**:314. 1915). In 1924, however, having in the meantime determined many hundreds of herbarium specimens of *Bidens heterophylla* Ort., I found immediately on reexamination that AITON's type was merely a cultivated form of that species. This surprising discovery led to a careful search through the remains of the single flowering, worm-eaten head. Two aristae were extracted and these both were retrorsely barbed. Such aristae are entirely unknown in the species from the southeastern United States, but are typical for *B. heterophylla* Ort.[19] Obviously the name *aurea*, published by AITON nine years prior to ORTEGA's *heterophylla*, and transferred to *Bidens* by me more than a decade ago, must be considered the valid trivial name for this well known species.

BIDENS MITIS (Michx.), comb. nov.; *Coreopsis mitis* Michx. Fl. Amer. Bor. **2**:140. 1803; *C. arguta* Pursh Fl. Amer. Sept. **2**:567.

[18] There is nothing mysterious about the AITON material being in this institution rather than at Kew. The AITON plant had been carried, without doubt, to BANKS or SOLANDER, then got into the Banksian Herbarium, and thus finally found its way, with other Banksian specimens, into the British Museum of Natural History (*cf.* JAMES BRITTEN, Jour. Bot. **50** [supplem. 3]:15. 1912).

[19] Another specimen was found in the same herbarium and coming from the old Chelsea Garden. It had the number 3417 and a copy of the original description of *Coreopsis aurea* was upon the label. It had the same peculiar aspect as the AITON type, so much so that it might well have been gathered from the same plant. All of its achenial aristae were retrorsely barbed.

1814 (*non* H.B.K.); *C. ambigua* Nutt., Jour. Acad. Philad. 7:75. 1834; *Diodonta mitis* Nutt., Trans. Amer. Phil. Soc. ser. II. 7:360. 1841; *D. leptophylla* Nutt., *l.c.; Coreopsis aurea* var. *subintegra* Torr. and Gray Fl. N. Amer. 2:339. 1843; *C. aurea* var. *leptophylla* (Nutt.) Torr. and Gray, *l.c.*, *C. aurea* var. *incisa* Torr. and Gray, *l.c.; Bidens coronata* var. *leptophylla* (Nutt.) Mohr, Contrib. U.S. Nat. Herb. 6:808. 1901; *B. aurea* var. *leptophylla* (Nutt.) Sherff, Bot. Gaz. 59:316. 1915.[20]

As already stated, the name *Bidens aurea* (Ait.) Sherff, heretofore considered as pertaining to a species from the southeastern United States, is found to belong to the Mexican species described by Ortega as *B. heterophylla*. Michaux's name *Coreopsis mitis* is found to be, in reality, the first name published for the former species, which is the species to be considered here. Torrey and Gray (*l.c.*) divided this species into the three varieties, *subintegra*, *leptophylla*, and *incisa*, on the basis of the amount of leaf division. But in this species the variation in leaf division is so fickle that it is not worth while to attempt the maintenance of varieties. Michaux described the leaves but scantily ("foliis petiolatis; infimis duplicato-pinnatifidis; supremis lineari-tripartitis"). I have before me, however, a specimen of his original material (Herb. Berl., *ex* Kunth, to whom it had been given by A. Richard in 1827). Its leaf segments are slender and fit the var. *leptophylla* better than the var. *incisa*, to which latter Torrey and Gray referred it.

Coreopsis ambigua Nutt. is represented by the type, still extant (Herb. Brit. Mus.). It is of the form described by Torrey and Gray as var. *subintegra*.

Bidens pilosa var. radiata Schz. Bip. in Barker-Webb and Berth. Hist. Canar. III. 2^{II}:242. 1836–50; *Coreopsis alba* L. Sp. Pl. 908. 1753; *C. leucanthema* L. Amoen. Acad. 4:291. 1759; *Bidens dondiaefolia* Less., Linnaea 5:155. 1830.[21]

In a former article (Sherff, Bot. Gaz. 64:32. 1917), I stated reasons for believing that *Bidens dondiaefolia* Less. was synonymous with *Coreopsis alba* L. Recently I have found Lessing's type (Herb. Univ. Halle). That plant matches exactly the Mueller specimen (no. 148, Herb. N.Y. Bot. Gard.) formerly cited by me, thus confirming very definitely my earlier treatment.[22]

[20] We may mention also the name *Leidon mite* (Michx.) Shuttl., which was printed on Rugel's label for a specimen of this species (Herb. Berl.), with *Coreopsis mitis* Michx. cited as a synonym.

[21] Numerous other synonyms must be omitted here.

[22] It may be added that in recent years studies have convinced me conclusively that *Coreopsis alba* L. and *C. leucanthema* L., both of them really *Bidens*, were synonymous and to be referred to varietal rank under *Bidens pilosa* L. While many botanists (O. E. Schulz, Otto Hoffmann, etc.) have already employed this varietal rank for the radiate plant in question, it appears that Schultz Bipontinus was the first to do so, using the name *radiata*.

BIDENS JACKSONII (S. Moore), comb. nov.; *Coreopsis Jacksoni* S. Moore, Jour. Linn. Soc. 35:347. 1902; *Bidens spathulata* Sherff, BOT. GAZ. 76:149. *pl. 13*. 1923.

My recent examination of the type specimen of *Coreopsis Jacksoni* S. Moore (Herb. Brit. Mus.) showed that it was merely a tiny, dwarfed specimen (*cf.* Moore, *l.c.*, "unfortunately a mere scrap") of the species described and illustrated by me under the name of *Bidens spathulata*.[23] Because, however, of its very diminutive stature (being only a few centimeters high), it lacked the spathulate type of leaves that so strongly characterizes larger plants. The leaves were more rotund in outline. For these reasons, MOORE's description of *Coreopsis Jacksoni* was, of necessity, so misleading that only a personal study of his type plant could impart an understanding of its specific status. As to its generic status, its complete lack of mature achenes, as also in the case of the type of *Bidens spathulata*, makes it necessary to rely upon general habit, which is more that of *Bidens* than of *Coreopsis*.

BIDENS PILOSA L. Sp. Pl. 832. 1753; *B. reflexa* Link Enum. Hort. Berol. 2:306. 1822.

The name *Bidens reflexa* Link has been variously construed by botanists during the past century. This is doubtless due to the fact that more than one species of plant was obtained in European botanical gardens from supposedly *Bidens reflexa* achenes which had been sent out from Berlin. Thus, some of the *B. reflexa* material in the Berlin Herbarium is *B. pilosa* L., while in the Herbarium of the University of Halle, for example, a specimen labeled "*Bidens reflexa* Hort. bot. Berol." is really *B. grandiflora* Balb. Fortunately, however, there exists in the Berlin Herbarium an original specimen by LINK himself. Its main label says, in pencil, "*Bidens* sp. Mexico." In ink is written, further, "*reflexa* m. (Link)." Another label says, "*Bidens reflexa* Link En. 2. p. 306. Hort. Bot. Berol." This specimen is clearly of LINK's type material, and matches his description, except that he erred in calling it a perennial, for it is an annual. It is nothing more than plain *B. pilosa* L., to which it must be referred.

BIDENS TRIPARTITA var. REPENS (D. Don), comb. nov.; *B. repens* D. Don Prodr. Fl. Nepal. 180. 1825; *B. trifida* Buch. in Roxb. Fl. Ind. Edit. II. 3:411. 1832; *B. Taquetii* Lévl. *et* Vant. *in* Lévl., Bull. Acad. Internat. Geogr. Bot. 20:3. 1910; *B. minuscula* Lévl. *et* Vant. in Lévl., *l.c.*—Saepius tantum 1–4 dm. alta, foliis nunc simplicibus et anguste lanceolatis, nunc tripartitis segmentis anguste lanceolatis vel saepe cuneato-lanceolatis. Achaenia cuneato-linearia, brunneo-subnitida vel subnigra, marginibus laevia vel aegre retrorso-hamosa,

[23] The type of *Bidens spathulata* was collected upon Mt. Kenia, British East Africa. The type of *Coreopsis Jacksoni* was collected in the small Kikuyu area immediately to the southwest.

corpore plerumque 5–8 mm. longa, apice 2- vel 3-aristata aristis retrorsum hamosis.

Bidens Taquetii Lévl. et Vant. and *B. minuscula* Lévl. et Vant. were described from specimens collected by TAQUET in Corea. *B. Taquetii* (*Taquet* 1035) is described as having radiate heads, the ligules being 3-striate. The several cotypes examined by me (Herb. Kew, Herb. Berl., etc.) uniformly lack rays, but even were tiny rays present they alone could not be of conclusive value in diagnosis, since true European *B. tripartita* has been known for many years to produce, in rare cases, small rays. *B. minuscula* (*Taquet* 1031) is merely a dwarfed form of *B. Taquetii.* Its several cotypes studied by me (Herb. Berl., Herb. Kew, Herb. Univ. Munich) excel, however, in having mature achenes. These have the slender, cuneate-linear, often almost glossy bodies with smooth margins, met with in a number of specimens from the Orient. Quite generally in herbaria this far-eastern form has been referred to *B. tripartita.* The dwarfed plants of it, however, do not resemble the dwarfed plants of *B. tripartita* commonly found, for example, in Europe. Rather do they have slender, oblanceolate leaves. Among the larger plants, however, various intergradations in foliage are found, particularly in Japan and Formosa, between the form of the Orient and the one typical in Europe. We have also the fact that in the particular oriental form under discussion the smooth margins of the achenes are strikingly different from the spinulose margins of normal *B. tripartita* achenes. In view of these considerations, it seems wisest to treat the TAQUET plants as representing a geographic variety.

Reference to literature shows that long ago this eastern form was listed for Nepalia (India) by ROXBURGH, who published the manuscript name *B. trifida* previously given it by BUCHANAN, and gave a full description. ROXBURGH'S description of the achenes ("flat, wedge-shaped, smooth, without angles; horns two, rarely three, backwardly hispid, diverging") is significant.

In the British Museum of Natural History are two old sheets, labeled in pencil, "*B. repens* Don." The plants are similar to the cotypes of *B. Taquetii* Lévl. et Vant., and match the description of *B. trifida* Buch. *ex* Roxb. They match also DON'S earlier but less ample description, published in 1825, of *B. repens.* At least one plant was collected by WALLICH, likewise in Nepalia, and bears WALLICH'S list no. 3187*a*. DON'S text included plants "*a D. Wallich nuperius missae*," and it is probable that he had seen these sheets before publishing his description of *B. repens.* In any case, however, his description rested primarily, as did ROXBURGH'S, upon the material collected by BUCHANAN[24] in Nepalia, and thus, with *B. repens* Don, *B. trifida* Buch. *ex* Roxb. is seen to be synonymous.[25]

[24] Known in literature also as FRANCIS HAMILTON (*cf.* DON, *l.c.;* Alph. DC. Phytogr. 418. 1880.)

[25] *V. Komaròv* (=*L. V. Komarow*) 1535, collected along the Mu-dan-dsian River, Province Kirinensis, Manchuria, September 19, 1896 (Herb. Berl., Herb. Brit. Mus., etc.) is a small slender mud form with elliptic or oblong-lanceolate leaves, and achenes

BIDENS TRIPARTITA var. ORIENTALIS (Velen. *ex* Bornm.), comb. nov.; *B. orientalis* Velen. *ex* Bornm., Bot. Centralbl. **36**:61. 1888 (*nomen*); Velen. in Sitzb. Boehm. Ges. Wiss. **1888**:48. 1889; Fl. Bulg. 250. 1891.—Var. achaeniis parvis, obovato-cuneatis, planis, nitidis, purpureo-nigris vel saepius nigrescentibus, ad margines retrorsum tuberculato-hamosis, alibi glabris vel versus apicem sparsissime pilosiusculis, corpore exterioribus 4–4.5 mm. longis et 2.2–2.5 mm. latis interioribus circ. 4.5 mm. longis et 1.5–2 mm. latis, omnibus biaristatis vel saepe imperfecte triaristatis, aristis retrorsum hamosis, duabus principalibus circ. 2 mm. longis.

The most distinctive feature of the several specimens examined from the type region is the small, often black achenes. Numerous intergradations between these and typical *B. tripartita* achenes are found in European material, however, nor in any other characters can I find sufficient constancy to warrant specific segregation from *B. tripartita* L. Evidently it is more logical to rank these small-fruited extremes, especially common in Bulgaria and Servia, as a variety of *B. tripartita* L.

BIDENS TRIPARTITA var. HIRTA (Jord.), comb. nov.; *B. bullata* L. Sp. Pl. 833. 1753; *B. hirta* Jord. in Gren. *et* Godr. Fl. Fr. **2**:168. 1850; *B. tripartita* sub-var. *rugosa* Coss. *et* Germ. Fl. Par. edit. II. 487. 1861; *B. bullata* var. *hirta* (Jord.) Coste Fl. Fr. **2**:357. 1903; *B. fastigiata* var. *hispida* Car. et St.-Lag. Et. fl. 459, *fide* Rouy Fl. Fr. **8**:219. 1903; *B. tripartita* var. *genuina* sub-var. *rugosa* Coss. et Germ. in Rouy *l.c.* 218; *B. tripartita* subsp. *bullata* (L.) Rouy and subsp. *bullata* var. *hirta* (Jord.) Rouy *l.c.* 219; *B. bullata* vars. *typica* and *glabrescens* Fiori Fl. Anal. **3**:302. 1904.

LINNAEUS presented a rather distinctive description of his *Bidens bullata*, but gave its habitat as in America. The single well preserved specimen in the Linnean Herbarium matches the original description very closely. There can be no doubt at all that LINNAEUS drew his description either from this specimen or from another identical with it. However, the plant is seen at once to be a European form, entirely unknown from America or any other continent than Europe. The plate of *B. bullata* published by HARDUIN (Animadv. Bot. *pl. 18.* 1764) differs in no important respect except that the principal leaves are undivided, instead of being tripartite with the lateral lobes small and inconspicu-

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moderately retrorse-hooked on margins. It was distributed under the name *B. tripartita* var. *limosa* Komaròv. The plant is an intermediate form between our var. *repens* and the species proper.

ous, as in LINNAEUS' specimen. The various herbarium specimens distributed by JORDAN from the vicinity of Lyon, under his name *B. hirta*, have the leaves undivided, but certainly cannot be said to differ consequentially from LINNAEUS' plant. In fact, other specimens have been collected in the same locality that have the leaves tripartite, just as in the Linnaean plant (e.g., *Boullu* 310). The variation in the amount of roughness is likewise considerable. In view of this variation in leaf division and in amount of roughness, there can be no question that the plants of LINNAEUS and of JORDAN are specifically identical.

On the other hand, these plants are connected by many apparently freakish specimens[26] with *Bidens tripartita*, and doubtless are to be regarded more accurately as a rather well marked foliage form of that species. ROUY (*l.c.*) appears to have come to a similar conclusion, but his adoption of subspecific rank for *B. bullata*, and his making of *B. hirta* a variety of the subspecies are too unconventional to secure wide acceptance. It happens, however, that COSTE (*l.c.*) definitely published the name *hirta* as a variety, although under the species *B. bullata*(!).[27] In this curious way the name *hirta* was the first to receive publication with varietal rank, and, under the Vienna Code, must be adopted instead of *bullata*.

It may be noted that the Abbé BOULLU, whose collections of this variety extended over more than thirty years, paid particular attention to the matter of variation in its leaves. Finding various forms intermediate between *B. hirta* Jord. and *B. tripartita* L., and having assumed that *B. hirta* as well as *B. tripartita* was a valid species, he suspected the intermediate forms of being hybrids.[28] In the genus *Bidens* hybridity is so rarely encountered that, in the absence of more positive evidence, the intermediate forms would seem best accounted for on the basis of ordinary variation. However, BOULLU described (Bull. Soc. Bot. Lyon. ser. II. 7:6. 1889) two of these supposedly hybrid forms under the names *B. hirto-tripartita* Boullu (with leaves broad, often tripartite) and *B. tripartito-hirta* Boullu (with leaves narrow, but likewise often tripartite).

[26] Especially branches from more or less injured stems.

[27] The manifest unwillingness of so many writers to unite the names *hirta* and *bullata* as synonyms surely must have been because LINNAEUS erroneously cited America as the home of *bullata*, and, further, because they themselves had never seen the Linnaean Herbarium specimen. Had they seen this plant, they would have been quick to realize that it was positively the form found in Europe and nowhere else.

[28] "Dans la Flore de France de Gr. et God. il est dit des feuilles de cette espèec ovales, fortement dentées, ce qui semble exclure toute idée de division; or parmi les exemplaires qu j'ai récoltés prés de la Bourbre à Pont-de Chérui, bon nombre portent quelques feuilles bi ou tripartites tantôt dans le haut, tantôt dans la bas de la plante. J'ai trouvé là le *Bidens hirta* comme perdu dans une forêt de *B. tripartita* L., atteignant près de deux mètres de hauteur. Les sujets à feuilles divisées seraient-ils un produit hybride de ces deux espèces? Sur les pieds isolés que j'ai rencontrés en plusieurs autres lieux, je n'ai pas observé cette division des feuilles." (From a printed clipping attached to sheet in Herb., Boiss.)

For the first of these two names, ROUY (Fl. Fr. **8**:219. 1903) has proposed the name, x *B. Boullui* var. *subbullata;* for the second, x *B. Boullui* var. *subtripartita.*

COSSON and GERMAIN'S *B. tripartita* sub-var. *rugosa*, a plant found about Paris, is seen from its description[29] to belong to this variety. I find no authentic specimen in the large Cosson herbarium (in Herb. Paris), but the main herbarium at Paris has a specimen from the environs of Paris (Charenton, on the islands of the Seine River, collected in 1841) given by WEDDELL in 1848 and clearly the source of COSSON and GERMAIN'S sub-variety. It is a remarkably hispid-scabrous form. In fact, its extreme roughness had been noted by WEDDELL himself, for his label reads, "*Bidens tripartita* L. var. *B. rugosa.*" Evidently, then, the name sub-var. *rugosa* was later derived from this. COSSON and GERMAIN'S comment, "Assez commun," is doubtless inaccurate, for such extremely rough forms appear rare in the vicinity of Paris.

BIDENS ANGUSTISSIMA var. LINIFOLIA (Schz. Bip. *ex* Klatt), comb. nov.; *B. linifolia* Schz. Bip. *ex* Klatt, Flora **68**:203. 1885.—A specie differt foliis indivisis, saepius 1–2 mm. latis.

In a former paper (SHERFF, BOT. GAZ. **64**:34. 1917) I have discussed the status of this form, predicting that it would probably be found to be a simple-leaved state of *B. angustissima* H.B.K. Further examinations of material have shown that in no way can the two be regarded as separate species. However, it does seem best to accord the simple-leaved form the rank of a variety under *B. angustissima.* Only very seldom is material found in herbaria that is not readily referable to one or the other.

BIDENS ASYMMETRICA (Lévl.), comb. nov.; *Lipochaeta asymetrica* Lévl., Fedde Repert. Spec. Nov. **10**:122. 1911.

This species belongs in no way to the genus *Lipochaeta.* It is a close relative of *Bidens sandvicensis* Less., from which it differs, however, in having a larger proportion of simple leaves (at least in the type material), also achenes which are strongly contorted and which are glabrous on the lateral margins as well as the faces.

Bidens brasiliensis, sp. nov.—Herba perennis, erecta, simplex vel supra ramosa, plus minusve tomentosa, ±1 m. alta. Folia principalia sessilia, indivisa, rhomboideo-elliptica, utrinque sensim angustata, membranacea, molliter denseque tomentosa, acriter numerosis dentibus serrata, 6–9 cm. long et 2.5–4 cm. lata; folia ramorum linearia, minora. Capitula subnumerosa (±40), discoidea, florescentia ±8 mm. alta et ±6 mm. lata, demum cum fructibus circ. 1.3 cm.

[29] "Tiges et rameaux rugueux-scabres, parsemés d'aiguillons sétiformes courts. Feuilles ord. rudes-scabres."

longa et 1–2 cm. lata. Involucri glabrati bracteae exteriores 5–8, lineares, subacute indurato-apiculatae, 3–6 mm. longae, interiores lanceolatae 7–10 mm. longae. Paleae achaeniorum corpora aequantes vel superantes. Achaenia atra, linearia, tetragona, omnino 8- (quaque facie 2-) sulcata, glabra vel supra sparsissime erecto-setosa, corpore 1–1.3 cm. longa et circ. 1 mm. crassa, ad apicem erecto-setosa et biaristata, aristis calvis vel saepius 1–3 hamis minutis retrorsum hamosis, usque ad 2.3 mm. longis, interdum deficientibus.

Auguste de Saint Hilaire 1196, State of Minas Geraes, Brazil (1816–1821; type in Herb. Paris).

In general habit *B. brasiliensis* resembles simple-leaved plants of *B. Riedelii* Baker and *B. Chodati* Hassler. From the former it differs in its more numerous heads, its lack of ray-florets, its elongate, aristate achenes, etc.; from the latter it differs in its wider and more rhombic leaves, more numerous heads, smaller involucres with much more glabrous and less numerous bracts, etc. The type had been collected by SAINT HILAIRE in two portions representing separately the lower, leafy stem and the branched, fruiting top. Both of these are taken as the basis for the description.

Bidens nyikensis, sp. nov.—Herba, verisimiliter perennis, caule simplici forsan ramoso, angulato, glabro vel ad summam piloso, ±3 dm. alto. Folia petiolata petiolis ciliatis anguste alatis 2.5–3.5 cm. longis, petiolo adjecto 4–11 cm. longa, pinnata, 3–5-partita, foliolis parce membranaceis, late linearibus vel rhomboideo-lanceolatis, 0.2–1.8 cm. latis, integris vel 1–2-lobis lateralibus instructis, margine revolutis spinuloso-ciliatisque, omnibus segmentis apice acutis. Capitula solitaria, terminalia, moderatim pedunculata pedunculo piloso usque ad 6 cm. longo, radiata, pansa ad anthesin ±3 cm. lata et ±9 mm. alta. Involucri hispidi bracteae exteriores circ. 8, moderatim vel late lineares, apice subacutae, 7–9 mm. longae et 1.2–2 mm. latae, interiores lanceolatae circ. 9–11 mm. longae. Flores ligulati circ. 8–10, flavi, tantum immaturi visi. Achaenia atra, lineari-oblonga, obcompressa, duabus faciebus non nisi ad summam hispida sed quaque circ. 8 sulculis lineata, margine apiceque valde erecto-ciliata, corpore 6.5–8.5 mm. longa et 2–2.7 mm. lata, biaristata aristis tenuibus, supra sparsissime infra saepe dense erecto-hispidis, 2–3 mm. longis, palearum apices coloratos parce superantibus.

A. Whyte 191, at altitude of 6000–7000 feet, Nyika Plateau, British Central African Protectorate, June, 1896 (type in Herb. Kew).

BIDENS RUWENZORIENSIS (S. Moore) Sherff, BOT. GAZ. 59:309. 1915; *Coreopsis ruwenzoriensis* S. Moore, Jour. Linn. Soc. 35:345. 1902.

The type, *G. F. Scott Elliot* 7410, was stated by MOORE to have been collected at Mt. Ruwenzori, but the original field-tag on the cotype at Kew gives, as the type locality, Kavirondo, some 400 kilometers to the east.[30] Both the type (Herb. Brit. Mus.) and the single cotype (Herb. Kew) consisted merely of fragments from the inflorescence. Thus the original description was necessarily very faulty and misleading as to general habit, size of leaves, etc. Fortunately, there have been received at Kew several additional specimens from the same general region: *A. Whyte*, from Nandi to Mumias etc., British East Africa, in 1898, 3 sheets; *J. D. Snowden* 28, at altitude of 4700 feet, Kyaka, Toro, Uganda, Brit. E. Afr., in 1913; *W. Small* 1192, short-grass land, foothills of Mt. Elgon at altitude of 5000 feet, Brit. E. Afr., November, 1914. These show the species to be a tall, handsome perennial, doubtless reaching a height of 2 meters, although SMALL reported it as growing 2–3 ft. As the mutual identity of all these specimens admits of no doubt, I have taken advantage of them to draw up a fresh and amplified description of the species:

BIDENS RUWENZORIENSIS (S. Moore) Sherff, descript. amplific. —Herba erecta, elata, verisimiliter 0.6–2 m. alta, caule glaberrimo vel ad summam interdum minute pubescenti, ramis validis, tereti-tetragonis, adscendentibus. Folia sessilia, per paria plus minusve connata, oblonga vel oblongo-oblanceolata, firme membranacea, albido-virescentia vel glaucescentia, glabra vel faciebus obscure pubescentia et margine ciliata, subgrosse subsimpliciterque serrata dentibus saepius indurato-apiculatis, principalia 10–13 cm. longa et 3–4.5 cm. lata. Capitula magna ramulos singillatim coronantia, radiata, pansa ad anthesin plerumque 7–8 cm. lata et 1.5–2 cm. alta. Involucri bracteae 3-seriatae, glabrae vel minute pubescentes, extimae (circ. 5) ovato-lanceolatae, foliosae et interdum sparsissime denticulatae, apice subacutae, usque ad 3 cm. longae et 1.5 cm. latae; interiores manifeste breviores, lanceolatae, margine non decoloratae. Flores ligulati circ. 12, lutei, ligula anguste ovato-oblongi, apice undulati vel circ. 3-denticulati, 2.3–3.5 cm. longi et 1–1.5 cm. lati; paleis lineari-lanceolatis, achaenia facile superantibus, 1–1.5 cm. longis. Achaenia atra vel atro-grisea, maxime obcompressa, oblonga, basi levissime angustata, margine apiceque erecto-

[30] The name *ruwenzoriensis* was not entirely inappropriate, however, since Mt. Ruwenzori was Elliot's destination, and his herbarium labels do in fact bear the heading, "Ruwenzori Expedition."

setosa, duabus faciebus plus minusve erecto-setosa et quaque circ. 8-sulcata, corpore 6–9 mm. longa et 1.8–2.3 mm. lata, biaristata; aristis tenuibus, maximam partem nudis sed versus basim 1–3 setulis erectis instructis, 1.5–2.8 mm. longis.

BIDENS CORIACEA (O. Hoffm.), comb. nov.; *Coreopsis coriacea* O. Hoffm., Engler Pflanzenw. Ost-Afr. 414. 1899.

The type (Herb. Berl.) was collected by FISCHER, no. 367, Massai Plateau, British and German East Africa, March 26–30, 1886. It consists of seven small fragments from the inflorescence. These offer a superficial resemblance to *Bidens ruwenzoriensis*. Indeed, S. MOORE (Jour. Linn. Soc. 35:345. 1902) appears to have suspected the possible identity of the two species. In *B. coriacea*, however, the outermost involucral bracts, as noted by MOORE, are not noticeably longer than the inner ones, and the achenes are mainly triaristate. In *B. ruwenzoriensis* the outermost bracts are easily the longest and the achenes are biaristate. Apparently it is wisest to await further specimens of *B. coriacea* from Massai before trying to equate the two species.

Bidens Rogersii, sp. nov.—Herba perennis, erecta, glabra, simplex forsitan ramosa, ±5 dm. alta. Folia petiolata petiolis alatis 0.5–3 cm. longis, petiolo adjecto 4–8 cm. longa, pinnatim 3–7-partita, foliolis crassiusculis, margine revolutis et sparsim ciliatis, linearibus, lateralibus usque ad 5 cm. longis, terminali elongato usque ad 7 cm. longo, omnibus integris vel pinnatisectis dentibus (lobulis) linearibus patentibus. Capitula solitaria, longe pedunculata, forsitan radiata (nullis ligulis visis), involucro ad anthesin ±2.3 cm. lato et ±1.1 cm. alto. Involucri bracteae aequilongae, exteriores circ. 8, lineares, infra hispidae, apice acutae, 0.9–1.2 cm. longae; interiores ovato-lanceolatae, margine decoloratae, dorso glabratae vel parce hispidae. Achaenia submatura nigrescentia, lineari-oblonga vel lineari-oblanceolata, valde obcompressa, quaque facie circ. 8-sulcata, margine apiceque valde faciebus aegre erecto-setosa, corpore 6–8 mm. longa et 1.8–2.2 mm. lata, biaristata; aristis tenuibus, calvis vel sparsim erecto-setosa, 2–3 mm. longis.

Rev. *F. A. Rogers* 10046, Sakania, Belgian Congo, August 18, 1911 (type in Herb. Kew).

BIDENS SCHIMPERI **leptocera,** var. nov.—Herba erecta, ±6 dm. alta, verisimiliter annua, caule glabro, ramoso. Folia superiora (inferiora non visa) subsessilia, usque ad 6 cm. longa, bipinnatisecta segmentis linearibus vel lineari-lanceolatis, valde membranaceis,

sparsim hispidis, ciliatis, acriter dentatis, 1–5 mm. latis. Capitula tenuiter pedunculata pedunculis usque ad 6 cm. longis, radiata, pansa ad anthesin circ. 2.5 cm. lata et 6–8 mm. alta. Involucri bracteae moderate hispidae, exteriores 5–8, lineares, acriter indurato-apiculatae, 3–6 mm. longae, interioribus ovato-lanceolatis parce longiores. Flores ligulati probabiliter circ. 8 (in uno capitulo imperfecto tantum 5 observati), lutei, ligula elliptico-oblanceolati, apice obscure denticulati, circ. 8–10 mm. longi. Achaenia parva valde obcompressa, ovato-oblanceolata vel lineari-oblonga, exalata, atra vel atrofusca, quaque facie circ. 8-striata, marginibus erecto-ciliata, faciebus erecte plus minusve spinuloso-setosa, apice setis erectis valde et perspicue coronata, corpore tantum 3–4.5 (rarissime –5) mm. longa et 0.8–1.1 mm. lata, biaristata; aristis perspicue tenuibus, leviter arcuatis, solum apice retrorsum hamosis, 1.5–3 mm. longis.

C. F. M. Swynnerton 845 A. Kilossa, German East Africa, May 8, 1921 (type in Herb. Brit. Mus.).

Many species of *Bidens* are polymorphic as to foliage, and yet reasonably constant as to characters of flower and fruit. *B. Schimperi* (Pl. III), however, offers an amazing range of variation in all respects. Thus, in typical *B. Schimperi* the external bracts of the involucre become 10–17 mm. long, but in forms of the species (of which *B. acutiloba* Sherff must be considered one) these may become only 3–6 mm. long. Again, in typical material, the achenial bodies range in length from 0.9–2.2 cm., while in various forms these range as low as 3–6 mm. long. Oliver and Hiern, also Otto Hoffmann, in their work on the African flora, came to recognize a large number of these forms as merely variations of *B. Schimperi*, although to a novice these would seem separate species. In most cases it appears unwise to attempt here any segregation of these forms as varieties. The plant collected by Swynnerton is unique in so many respects, however, that it is thought best to give a full description and distinct varietal treatment.

Bidens speciosa Gardn. in Hook. Lond. Jour. Bot. 4:126. 1845; *B. pallida* Rusby, Bull. N.Y. Bot. Gard. 4:389. 1907.—Pl. IV.

Bidens pallida Rusby was founded upon *Miguel Bang* 2152, Coripati, Yungas, Bolivia, April 25, 1894 (Herb. Boiss.; Herb. Brit. Mus.; Herb. Copenh.; Herb. Deless.; Herb. Kew; Herb. Mo. Bot. Gard.; Herb. N.Y. Bot. Gard., type; Herb. Mus. Vienna; Herb. Univ. Vienna, etc.). The specimens were somewhat immature, but they are seen to be merely a foliage form of *B. speciosa* Gardn., a

species close to *B. squarrosa* H.B.K. and very common in Brazil, although in rare cases it has been found elsewhere (*Scherzer* 853, alt. 9100 ft., San José, Costa Rica, September–November, Herb. Mus. Vienna; *José Steinbach* 5584, Orilla del bosques, alt. 400 m., Rio Surutú Buena Vista, Province Sara, Dept. Santa Cruz, Bolivia, April 15, 1921, Herb. Deless., Herb. Field Mus.).

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EXPLANATION OF PLATES I–IV

PLATE I

Bidens Moorei: *a*, flowering and fruiting branch, ×0.68; *b*, exterior involucral bract, ×1.36; *c*, interior involucral bract, ×2.72; *d*, ligule, ×1.36; *e*, palea, ×2.72; *f*, disc floret, ×2.72; *g*, achene, ×2.72; *a–g* from *Gossweiler* 3339, type, in Herb. Brit. Mus.; *B. Moorei* var. *verrucosa;* *h*, *i*, achenes, ×2.3, from *Gossweiler* 3021, type, in Herb. Brit. Mus.

PLATE II

Bidens urophylla: *a*, flowering and fruiting branch, ×0.66; *b*, exterior involucral bract, ×5.3; *c*, interior involucral bract, ×5.3; *d*, ligule, ×5.3; *e*, palea, ×5.3; *f*, disc floret, ×5.3; *g*, achene, ×5.3; all from *Martius*, near Mariana, Minas Geraes, Brazil, type, in Herb. Univ. Munich.

PLATE III

Bidens Schimperi (forms of): *a*, *j*, flowering and fruiting specimens, ×0.64; *b*, portion of leaf from *a*, ×3.2; *c*, *k*, exterior involucral bracts, ×3.2; *d*, *l*, interior involucral bracts, ×3.2; *e*, *m*, ligules, ×3.2; *f*, *n*, paleae, ×3.2; *g*, *o*, disc florets, ×3.2; *h*, *p*, outer achenes, ×3.2; *i*, *q*, inner achenes, ×3.2; *a–i*, from *Volkens* 384, type of *Bidens acutiloba*, in Herb. Boiss.; *j–q*, from *Schilling* 67, Kilimanjaro district, Africa, in 1903, Herb. Berl.

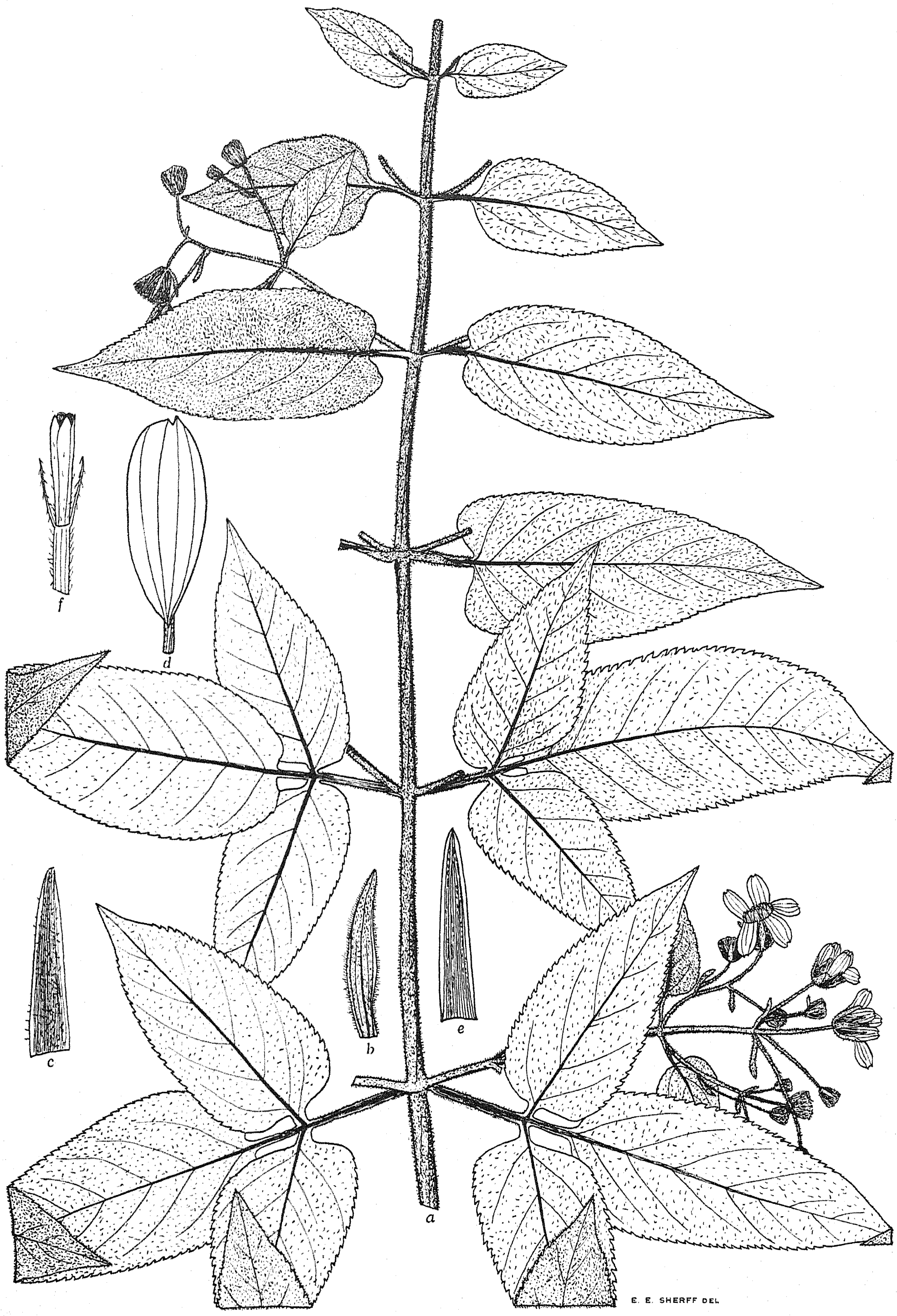
PLATE IV

Bidens speciosa (form of): *a*, portion of flowering branch, ×0.65; *b*, exterior involucral bract, ×3.3; *c*, interior involucral bract, ×3.3; *d*, ligule, ×2.6; *e*, palea, ×3.5; *f*, disc floret, ×3.5; all from *Bang* 2152, mainly the (type) specimen in Herb. N.Y. Bot. Gard.

SHERFF on BIDENS

SHERFF on BIDENS

SHERFF on BIDENS



SHERFF on BIDENS

ORIGIN OF CLEAVAGE POLYEMBRYONY IN CONIFERS[1]

JOHN T. BUCHHOLZ

(WITH PLATES V–VII AND TWO FIGURES)

Polyembryony and developmental selection

Polyembryony, as it occurs in gymnosperms, is the expression of a peculiar form of developmental selection. There are two forms of polyembryony with respect to the origin of the embryos: simple, due to the fertilization of several eggs; and cleavage, due to the splitting of the product of the individual zygotes.

In some conifers, such as *Pinus* (figs. 4, 6, 12), *Tsuga* (fig. 11), *Cedrus* (fig. 20), and *Libocedrus* (figs. 23, 24), cleavage polyembryony can be recognized both by counting the number of embryos produced from each zygote, and by tracing each embryo back to a single celled suspensor. Simple polyembryony is illustrated by *Abies* (fig. 7), *Picea* (fig. 21), *Larix*, and *Pseudotsuga* (fig. 14), where the suspensor is made up of four or more collateral elongated cells which may be traced back to the same archegonium, and there are usually only as many embryos as there were fertilized eggs. In all instances this polyembryony results in a struggle for existence within the developing seed (embryonic selection), in which sometimes only two or three, sometimes as many as thirty or forty, very young embryos are in competition. In this struggle, the embryo possessing the greatest growth activity soon gains an advantage, usually represented by its foremost position with respect to its competitors, and it eliminates all of them sooner or later, for practically always only one embryo remains in the mature seed. The elimination usually takes place in the earlier stages of development, so that by the time the largest embryo has begun to organize its cotyledons, there are only very small traces of the checked embryos remaining. Frequently the successful embryo may be recognized at an early stage, as the one foremost in position; soon it is the largest and comes to

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possess a longer and more multicellular suspensor than any of its competitors. In this elimination, chance plays a minor rôle, for the persisting embryo usually merits this reward. This is a conclusion based upon observations on a large series of embryos of different conifers, dissected out in various stages of development. It seems clear from such studies that the embryo possessing the greatest growth vigor usually wins in the competition. The more speedily an embryo becomes multicellular and of larger diameter than its neighbors, the sooner will it be able to push out a stiff multicellular suspensor, which is sometimes equipped with hooks or spurs formed by many outwardly projecting embryonal tubes (fig. 24). This suspensor is in reality an organ of competition; it is the structure by means of which the eliminated embryos are pushed back toward the micropyle, where they become starved and crushed.

The values measured by embryonic selection, expressed mainly as variations in their growth rate, represent differences which are inherent among the several embryos. These differences may have their origin in a chromosomal re-assortment of hereditary qualities during the first few cell divisions of the zygote, as was observed by HUTCHINSON (5) in *Abies*, or they may be due to inherent differences among the several zygotes, where embryos from several eggs enter into the competition.

In conifers, this developmental selection often measures more than the differences between the embryos, for it really consists of a relay race. The successful embryo plus the pollen tube which produced it must be superior to the sum of the pollen tube and embryo of its neighbors within the same ovule. Where cleavage polyembryony is involved, the competition measures the best pollen tube plus the best fraction of its zygote, against many other similar combinations within the same developing seed.

Direction of evolution of cleavage polyembryony

Some light is thrown on the nature of cleavage polyembryony by considering the direction in which its evolution must necessarily proceed. Let us first consider the possible outcome if a plant having simple polyembryony should give rise to the condition of cleavage polyembryony. Could cleavage polyembryony originate and survive

under conditions as they exist within an ovule, as it must be assumed that it has in relatively recent time, if cleavage polyembryony is a feature of recent origin?

Suppose, for example, that in fig. 1, representing a condition similar to *Pinus*, cleavage polyembryony does not exist. Let *B* represent in this instance a condition in which the entire zygote forms the embryo, and in which the competition which exists is between *B* and *A*, or other neighboring zygotes. Let *A* represent a

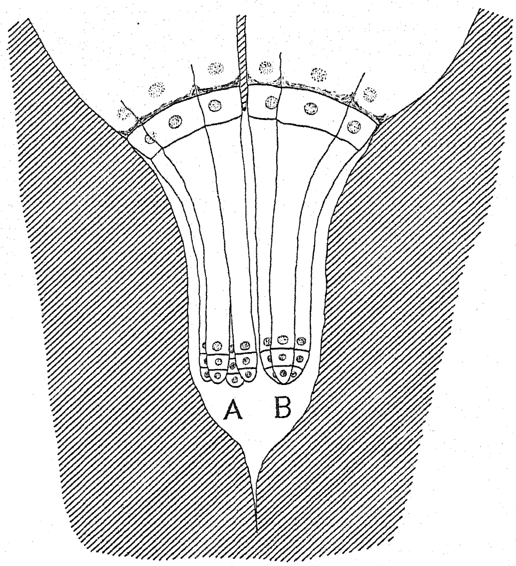

FIG. 1.—Two conditions in embryogeny (explanation in text).

departure from the usual condition, a change in which cleavage of the zygote is taking place. Under these conditions, what chance is there that any of the several embryos at *A* might survive? Judging by the available facts about the competition among conifer embryos, the answer is not difficult; there is little or no chance that any of the smaller embryos at *A* might survive. Granted that fertilization of the two eggs was simultaneous, and other conditions except cleavage polyembryony the same, since embryo *B* is so much larger than *A* to begin with, and since it starts its forward growth with a 4-celled and much more rigid suspensor (in fact it has in the number of its

cells a fourfold advantage over the embryos at A), there can be no doubt of its supremacy from the beginning. Embryo B will probably contribute the successful embryo to the seed, and the change to cleavage polyembryony in A will leave no progeny.

Suppose that A represents the usual condition, that to begin with cleavage polyembryony is present as shown here, and that a change from the condition in A to that in B has taken place. This time the change will survive, for the new condition B is the larger embryo, equipped with the stiffest suspensor, while the several units of A will lose out in the competition with B. Thus the new condition represented by B would survive the embryonic competition to produce the seed germ, and certainly it would leave a progeny, which could continue and establish this characteristic of simple polyembryony, although its derivation was from a condition of cleavage polyembryony. It is therefore not likely that cleavage polyembryony has had its genesis in relatively recent times, but rather that this condition had its origin possibly as early as the time of the origin of seeds themselves. At least it must have appeared before the mechanism of developmental selection reached its present adjustment; before the combined activities of pollen tubes plus their respective whole embryos from separate zygotes were pitted against each other in the decisive internal competition which exists within the ovules of conifers. It seems clear to the writer that embryonic selection would permit evolution to proceed from cleavage polyembryony to simple polyembryony, but not in the reverse direction.

The evolution of simple polyembryony from cleavage may be considered a kind of orthogenesis, but one which can be explained on a mechanical basis; it is a question of embryonic selection. A very definite product of this orthogenetic mechanism is a very large suspensor, which is certainly an over developed structure when we consider the structure and function of suspensors generally in other seed plants, where embryonic selection is not usually found.

Failure of simultaneous fertilization

It follows from the nature of this developmental selection, that the origin of cleavage polyembryony is to be sought under conditions in which only a single egg was fertilized, or in which the fertili-

zation of several eggs was not simultaneous or nearly so, with the result that the embryonic selection did not eliminate the smaller products of cleavage. This condition of successive rather than simultaneous fertilization would protect the much smaller and weaker embryos coming from the cleavage of an early zygote. Either of at least two conditions could have brought about successive fertilization or failure of simultaneous fertilization. Both have to do with the necessary conditions existing at the beginning of siphonogamy, and both may have played an important part at this stage in the origin of cleavage polyembryony.

Transition to pollen tubes

The first of these conditions which could preserve cleavage polyembryony against the odds of elimination through embryonic selection has to do with the change from fernlike microgametophytes to pollen tubes. It would seem probable that during the earliest stages of pollen tubes, when these male gametophytes were for example in a state of transition from that of heterosporous ferns to the condition found in pine, these pollen tubes were not equally able to grow in the sporophyte tissue. With free swimming sperms, as occurring in ferns, simultaneous fertilization of several eggs was the usual condition which initiated the process of embryonic selection, but with the change to pollen tubes, one would expect that simultaneous fertilization of two or more eggs would be only rare and very accidental.

The time of fertilization of neighboring archegonia probably varied by weeks or months, where this varies today in living forms by only days or hours. This interval of difference in growth rate of several relatively imperfect pollen tubes was the occasion for the survival of cleavage polyembryony, should it have originated either as a new mutation or in a segregation following some kind of hybridization.

The second of these conditions is suggested by a fragment of paleontological evidence. It also has to do with the beginnings of siphonogamy. Let us consider the seed of *Cordaianthus* as described by RENAULT (fig. 2). Here the nucellus is shown with a narrow passage which leads down to a pollen chamber; the microspores

are found wedged in this passage. Remembering that in *Pseudotsuga* and some other gymnosperms the pollen normally germinates in such a micropylar passageway, one can readily see that if a form similar to *Cordaianthus* should change to siphonogamy with such a disposition of structures, only one pollen tube at a time could function. The upper pollen grain is so much farther from the eggs than the lower one to begin with, that an equal length of these pollen tubes could not effect a simultaneous fertilization of the eggs in several separated archegonia. Whether the microspores begin to germinate in the micropylar passage or within the pollen chamber which is usually found below, their distances from the various archegonia are practically certain to be unequal. Thus the zygote resulting from the first fertilization might undergo cleavage, and the resulting smaller embryos become large enough to win out in competition against a later zygote having no cleavage, even though the second is a much larger embryo, one derived from an entire egg.

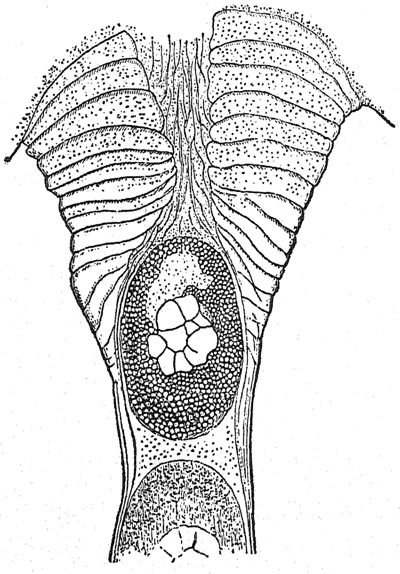

FIG. 2.—*Cordaianthus Grand'Euryi:* section of beak of nucleus; wedged in passageway are two large pollen grains, lower one showing part of surface of exine, and also multicell ularinterior; ×225 (from COULTER and CHAMBERLAIN, after RENAULT).

The seed of the form from which conifers were derived may have differed from that illustrated by *Cordaianthus*. There is no doubt, however, that the mechanism involved would be very similar in any megasporangium in which the microgametophytes became penetrating digestive structures. Whether the change was wrought by the one alternative or the other is of little importance; the stress should be placed on the fact that the newly formed pollen tubes did not effect fertilization of the eggs simultaneously. It is probable

that both of these conditions existed at this stage, and that both contributed in producing for a time a condition in which the several pollen tubes did not simultaneously fertilize the eggs of neighboring archegonia, thus permitting cleavage polyembryony to become an established feature of practically all primitive conifers, before they began yielding in their evolution to the mechanical tendency to return to simple polyembryony. This variability in the growth rate of pollen tubes would also serve to retain a species or an entire group in the condition of having cleavage polyembryony.

Cleavage polyembryony not recent

Considering the foregoing, it is difficult to explain how cleavage polyembryony could be a feature of recent origin. Orthogenesis, if we may be permitted to use this term in designating the mechanical tendency to pass into simple polyembryony, favors the elimination of cleavage polyembryony wherever pollen tube competition is effective enough. Cleavage polyembryony was therefore only an incidental event, accompanying or closely following the early advent of siphonogamy, and this is why many conifers have returned to simple polyembryony, especially in forms where the pollen tubes afford a competitive mechanism of a sufficient degree of perfection so that the fertilization of several eggs by competing pollen tubes is practically a simultaneous event.

Evidence from comparative embryogeny

The conclusion that cleavage polyembryony must have been a rather primitive condition, and one through which nearly all conifers have passed, was reached independently on morphological grounds. For example, in *Pinus* (1), which we regard as very primitive in its embryogeny, we have cleavage polyembryony associated with fern-like apical cell growth from the time of the first appearance of walls, but the apical cell usually disappears before the embryo cell mass contains 500 or more cells. In *Cedrus* and *Tsuga* we also have both cleavage polyembryony and apical cell growth, the latter apparently not for so late a period of development, although no adequate opportunities have permitted the writer to verify this point by means of serial sections. In *Picea*, *Larix*, and probably in *Pseudo-*

tsuga and *Abies*, there seems to be no normal apical cell growth at any stage, and here we usually have simple polyembryony. Thus in Abietineae, at least, cleavage polyembryony is associated with the fern type of apical cell growth in the earliest stages of embryogeny. From the writer's observations in Abietineae (2) it seems that both of these features were eliminated together. Among the other conifers usually regarded as less primitive, some have become specialized in the direction of cleavage polyembryony, and others by retaining apical cell growth. Probably some have retained both of these features in the embryo development, but exact knowledge of these details of conifer embryogeny is still very fragmentary.

Of course a primitive condition is frequently associated with advanced structures or conditions, and this mere association with the apical cell is not positive proof, but certainly the absence of apical cells among forms with cleavage polyembryony, and from the earliest stages (tracing each embryo back to a single cell of the proembryo), would constitute negative evidence. This negative evidence is absent, and to this extent at least we may utilize association of apical cell growth with cleavage polyembryony as suggestive of the primitive nature of the condition of splitting embryos.

The most convincing morphological argument favoring the primitive nature of cleavage polyembryony, however, comes from a study of the rosette embryos. These are the result of an extreme expression of cleavage polyembryony. The rosette cells in *Pinus*, for example, usually give rise to a group of four embryos, which send out suspensors; and in Abietineae, at least, the rosette embryos develop after the manner of the primary embryos by means of an apical cell. Figs. 6 shows an embryo complex representing the zygote from two neighboring archegonia. The eight primary embryos below have split apart as usual and are of good size, and in addition rosette embryos have developed from the rosette cells located at the base of the archegonia. In the group at the top to the right in fig. 6 (detail shown in fig. 25), several rosette embryos have pushed out suspensors which make them appear as very normal embryos. Were it not for their position with respect to the collapsed folds of the primary suspensor (*s*), these rosette embryos would easily pass for primary embryos. In the group of rosette embryos to the left (fig. 25 *re*) the

suspensors have not elongated, but all four of the rosette cells have undergone proliferation.

Fig. 3 represents a very much younger stage in *Pinus Laricio*, before the cleavage of the primary embryos has taken place. Fig. 4 is a stage in which the eight primary embryos from two zygotes may all be found. The rosette cells of one embryo system have not undergone development, but those of the other (*re* at the extreme left) have become multicellular. The latter are shown more highly magnified in fig. 5. Only in the later stages, such as figs. 4 and 6, do they grow out distinct embryonal tubes to form a suspensor, and frequently they abort in much earlier stages.

Even at their best, as in some species of *Pinus*, these rosette embryos are vestigial structures; they do not develop far. They are distinctly survivals from a condition of greater cleavage polyembryony. It is not probable that the rosette embryos in *Pinus*, or in any other conifer where they are found, ever contribute the embryo of the mature seed.

In *Cedrus* these rosette cells are usually persistent, and the rosette embryos frequently develop somewhat (figs. 16, 17), often becoming nearly as large as those of *Pinus* shown in fig. 4, but none were discovered with elongated suspensors. Figs. 18 and 19 illustrate well developed rosette embryos in *Cedrus libani*. *Cedrus* also has cleavage polyembryony, but the actual separation of the vertical rows of cells occurs only after one or two sections of embryonal tubes comprising the secondary suspensors have elongated quite fully. Fig. 16 shows such a stage in *Cedrus*, before cleavage has occurred among the primary embryos. In stages taken a week or ten days later, separation of the primary embryos had invariably taken place, resembling the *Pinus* embryos of figs. 4 and 6, and by this time a number of rosette embryos had usually begun to develop from the rosette cells. Fig. 20 is a single embryo of *Cedrus* on the end of a single celled secondary suspensor, after cleavage has occurred. A count of the number of primary embryos present in *Cedrus* at this stage, as well as the possibility of tracing the embryos back to their secondary suspensors of single embryonal tubes, easily distinguishes the embryo system of *Cedrus* as having cleavage polyembryony practically always, but with a distinct delay in the sepa-

ration of the embryos, as shown in fig. 16. That the development of the proembryo of *Cedrus atlantica* is practically identical with that of *Pinus* is indicated by the work of SMITH (9), who described and illustrated some of the stages.

In *Tsuga heterophylla*, as well as in *T. canadensis*, the rosette cells are found, but they abort early. They seem always to abort in the 1-celled stage without dividing further, although the separation of the four lower cells is a constant feature. Fig. 10 shows the rosette cells in an early stage of *T. canadensis*, and fig. 11 shows eleven of the primary embryos of *T. heterophylla* coming from three zygotes at a later stage. In the latter the rosette cells have collapsed so completely that they are difficult to demonstrate in a photograph, in fact they are not always easy to find in a preparation. Four primary embryos of *T. heterophylla* are shown in fig. 13.

In *Abies balsamea* the rosette cells are also found, sometimes as persistent nucleated cells (fig. 9). We agree with HUTCHINSON (6) that they are usually aborted, but in a count of 47 zygotes (of about the age shown in fig. 7) they were found in 12 per cent of the cases examined, where a clear view of the rosette was possible. This summary was made by counting the four vertical rows of cells separately, because frequently there were only one or two rosette cells which persisted in an embryo system, the other members of the rosette group having collapsed. Rosette cells were found more frequently in *Abies* than cleavage polyembryony. In 14 zygotes the rosette region was masked by the basal plate, and could not be observed satisfactorily. Only a few rare instances of rosette cells forming embryos were found in *Abies*. In fig. 8 the zygote to the right represents one of these, which is only 2-celled, and in the zygote to the left there are also a few undivided rosette cells. Usually the embryo presents the appearance shown in fig. 7, where the rosette cells have completely collapsed, and are represented only by their very thin walls, which may sometimes be found under high magnification.

While only 12 per cent of the rosette cells of *Abies balsamea* were actually persistent as living cells, in 40 per cent of the remaining cases their cell walls were discernible, although the protoplast had collapsed and often completely disintegrated. In the remaining

48 per cent of the cases, such little evidence of the rosette cells could be found that they might have been overlooked completely.

Abies alba also has rosette cells, according to SCHACHT (8), who shows three stages, all of them including the rosette cells, which do not appear to collapse as early as in *A. balsamea*. It is therefore certain that, except for the abortion of rosette cells, *Abies* and *Pinus* have homologous cells in the proembryo.

There is another point which should be mentioned in regard to an apparent difference between HUTCHINSON's studies and those of the writer. HUTCHINSON studied only the stages as they were found in sections; the writer necessarily confined his efforts to the dissectible material at a slightly later stage. If some of the early embryos observed by HUTCHINSON abort and disintegrate completely, they might not be included with the dissected embryos of a slightly later stage. Judging from serial sections alone, HUTCHINSON found cleavage polyembryony somewhat more frequent than the writer, who used completely separated suspensors as the criterion of cleavage. Our findings are in substantial agreement concerning the main facts; the actual formation of rosette cells, whether they collapse very early or not, and in the usual non-occurrence of cleavage polyembryony.

In *Picea excelsa* (fig. 21), *P. mariana*, and *Larix europea* the rosette cells are formed, although they also collapse without forming rosette embryos in the material which was observed. They are difficult to demonstrate in embryos which have produced 3 or 4 sections of embryonal tubes, such as those of fig. 21. In a small percentage of cases, single nucleated rosette cells were found to hold over into stages of the age represented by fig. 21, but none of them were found developed to the 2-celled stage (fig. 22 illustrates the usual appearance of the collapsed rosette of *P. excelsa*). Only a single instance of a separation of primary embryos on the ends of embryonal tubes was observed among hundreds of embryos of *P. mariana* and *P. excelsa* which were examined.

Pseudotsuga has embryos without the rosette cells, as shown in figs. 14 and 15. Here the uppermost cells are the elongated suspensor cells, and no free nuclei were observed in the remains of the egg above these suspensors. The upper ends of the suspensors may be seen to

extend up into the base of the archegonium (fig. 15). In fig. 14 three embryos have come from three of the four or five archegonia, and no cases of splitting of these primary embryos were found. The three primary embryos are on their secondary suspensors (e_1), and were all bent slightly to one side in mounting.

In this series of embryos of the Abietineae, the outstanding feature is the gradual elimination of the rosette embryos. Even in *Pinus* they are vestigial, in *Cedrus* more so, and in the other forms the aborting initial cells represented by the rosette group are the last remains of the former rosette embryos. These aborting structures in *Larix*, *Picea*, or *Abies* could not be considered as having given rise to embryos in forms where cleavage polyembryony never existed, but reading the series in the other direction, they present clear evidence of reduction from 8-parted to 4-parted cleavage polyembryony with completely aborting rosette cells as in *Tsuga*, to simple polyembryony with rosette cells (or rarely the embryos) sometimes persisting as in *Abies*; and finally to forms illustrated by *Larix* or *Picea*, where only the last vestiges of these structures remain in embryos having no cleavage polyembryony. *Pseudotsuga* is the only member of this group which shows no rosette cells as anatomical evidence of having passed through a condition of cleavage polyembryony.

In a previous paper (3), the writer has demonstrated the normal existence of rosette cells in *Cephalotaxus*, and there seems to be similar evidence of such a reduction from cleavage polyembryony, sometimes involving rosette embryos in some other Taxineae and in certain Podocarpineae which will be described later.

In *Libocedrus*, which is included here because of its rather extreme illustration of cleavage polyembryony, the walled rosette cells are not found, according to LAWSON (7), and it will be seen (fig. 23) that a view of the rosette region is not favorable in the dissected preparations. *Libocedrus* is a form in which cleavage polyembryony is developed as a specialization. Since each of the 13 or more embryos may be traced back to single embryonal tubes or suspensor cells, there can be no doubt of the separation of the several zygotes in the production of this embryo complex. A more detailed account of the embryogeny of several Cupressineae will be given by the writer in another paper.

In *Cephalotaxus* (3) the deciduous cap at the tip of the embryo may have been involved in the elimination of cleavage polyembryony for the primary embryo, and if this is a result generally where caps are formed (although not necessarily the present function of this structure), it is possible that even Araucarians may have passed through a condition of cleavage polyembryony. Thus we may reasonably suppose that practically all conifers which do not show cleavage polyembryony have passed through this condition in the past, some of them reaching a point of embryonic organization in which all structural evidence of this feature is completely suppressed.

Rôle of cleavage polyembryony

There is one other fact or coincidence of some significance in this connection. It was previously pointed out by the writer (4) that several forms of developmental selection are usually, if not always, found in the reproductive life cycles of vascular plants. Table I

TABLE I

NATURAL SELECTION Environmental process occurring in external physical and biological environment of organism, where conditions of struggle for existence are very complex	Struggle against unfavorable environment of physical surroundings Struggle against other species; extraspecific competition Struggle against fellows; intraspecific competition
	Selection between vegetatively branching parts of either the gametophyte or sporophyte; buds and branches of trees, which later give rise to reproductive parts
DEVELOPMENTAL SELECTION Occurring during early embryonic or gametophytic stages within tissues of parent plant, under conditions uniform for competing individuals	Interovular selection, between ovules within same ovary: (1) after fertilization, largely due to activities of contained embryos; (2) before fertilization, due in part to activities of contained female gametophytes, megaspores, or archesporial cells Embryonic selection, between embryos within the same ovule, or within tissues of parent gametophyte Gametophytic selection: (1) between male gametophytes, such as pollen tubes within carpellary and nucellar tissue; (2) between female gametophytes within the same ovule Gametic selection: (1) between male gametes or sperms; (2) between female gametes or eggs

shows the interrelations of these forms of developmental selection and of these to natural selection.

A glance at the summary of table I makes it evident that most, or in fact nearly all of the more important or better understood forms of developmental selection were rendered ineffective in the transition to seeds at the time when free swimming sperms were no longer liberated in a drop of water connecting them up with several eggs. At this stage in the evolution of seed plants the megasporangia were not clustered and crowded close together as they are in the ovary of a flower, so that the interovular selection was probably not found. It is not even likely that a compact strobilus had been developed, for the examples of Cordaitales whose strobili have been found are all comparatively loose or open structures. The compound nature of the conifer cone itself clearly suggests a reduction from something more open and complex.

The restriction in the swimming range of the sperms occasioned by siphonogamy eliminated simultaneous fertilization, and when the embryos of neighboring archegonia were not produced simultaneously, the embryonic selection could not measure the inherent differences in the rate of growth, etc., of the several zygotes. Pollen tubes had not been perfected, so that there probably was a stage in evolution of conifers in which very few forms of developmental selection existed; that is, in which the internal forms of selection in the life cycle were ineffective as forms of selection, or entirely absent. The forms of selection which were operative, if any, were forms of natural selection and clonal selection, which occur in the complex external environment where the effectiveness of selection is much less positive.

In seeking for a possible advantage gained from cleavage polyembryony, one having a survival value, the writer has wondered whether the advent of cleavage polyembryony at this stage might not represent an adjustment which restored a highly effective form of developmental selection to the life cycle. This would assign to cleavage polyembryony a sort of supplementary rôle, one which played an important part in making some kind of embryonic selection possible during the period when male gametophytes became pollen tubes.

Cleavage polyembryony occurs rarely in angiosperms, and no form of polyembryony serves such an important rôle in the higher seed plants, which have long and rapidly growing pollen tubes, and which also possess interovular and other forms of developmental selection. There seems to be little or no evidence in the development of the embryo indicating that angiosperms shared such a polyembryonic history in their embryogeny.

Summary

This discussion shows that cleavage polyembryony is a feature of the embryogeny which must have had its origin before the developmental selection within an ovule had become adjusted, under conditions when only one egg at a time was fertilized. It is possible that this occurred during the stage of transition from independent microgametophytes to pollen tubes, or it may have appeared after this time. In any event, it must have occurred before the present adjustment was perfected wherein pollen tubes take part in the process of developmental selection, and cleavage polyembryony is therefore a palingenetic character. The destructive competition which may be recognized in conifer embryos favors the development of stiff, extremely long suspensors, and also the reversion from cleavage to simple polyembryony. Morphological evidence is presented which supports the explanation that most of the living conifers whose embryogenies are known, perhaps all of them, have passed through a stage of cleavage polyembryony. The latter is therefore a primitive feature so far as conifers are concerned, although some of them have retained cleavage polyembryony as a specialization.

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EXPLANATION OF PLATES V–VII

The following lettering is used in all the figures: *s*, primary suspensor cells; e_1, embryonal tube or tubes of secondary suspensor; e_2, e_3, later embryonal tubes added to secondary suspensor (s, e_1, e_2, etc., produced by elongation of cells derived from basal end of embryo); *r*, rosette cells, group of 4 embryo initial cells located above primary suspensors (*s*); rosette cells (*r*) sometimes give rise to rosette embryos (*re*).

PLATE V

FIGS. 1 and 2 are text figures.

FIG. 3.—*Pinus Laricio:* two early embryos on primary suspensors before cleavage of zygotes; ×35.

FIGS. 4–6.—*P. Laricio:* each an embryo complex produced from fertilization of two eggs, with subsequent splitting of two zygotes into four primary embryos each, and development of rosette embryos; fig. 5 is enlarged detail of one of rosette embryos of fig. 4; rosette embryos of fig. 6 enlarged in fig. 25; ×35.

FIG. 7.—*Abies balsamea:* single embryo derived from one zygote (an instance where cleavage polyembryony does not usually occur); ×35.

FIG. 8.—Rosette cells of *A. balsamea* with a 2-celled rosette embryo at *re;* ×105.

FIG. 9.—Rosette cells of *A. balsamea;* ×90.

FIG. 10.—Early embryo system of *Tsuga canadensis*, showing presence of nucleated rosette cells at this stage (before primary embryos have separated); ×105.

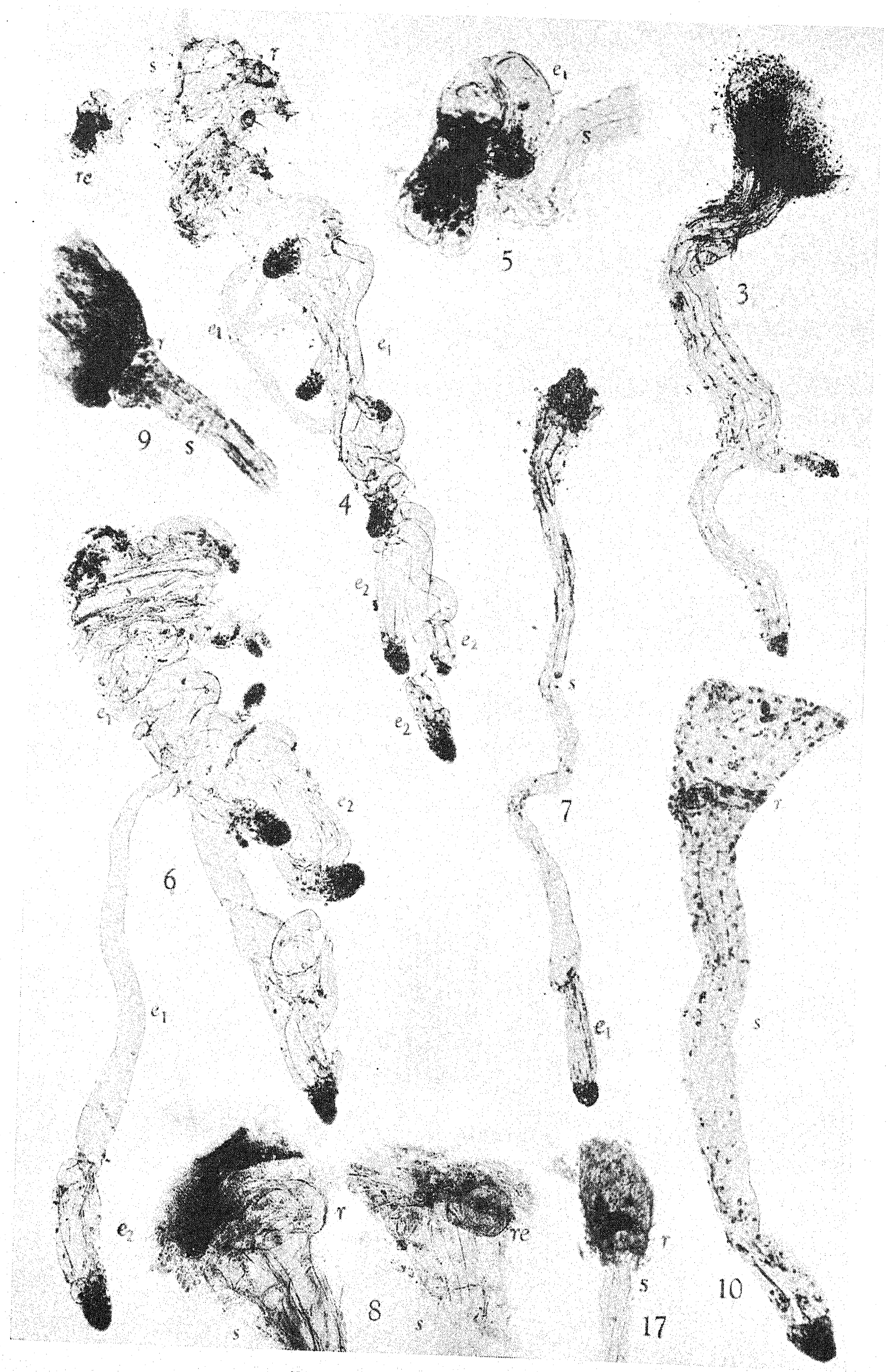
FIG. 17.—Detail of rosette cells of *Cedrus libani* during early stage; ×35.

PLATE VI

FIGS. 11 and 13.—*Tsuga heterophylla:* fig. 11 shows embryo complex with eleven of the twelve primary embryos derived from fertilization of three eggs; primary suspensors collapsed; each embryo is multicellular and has produced secondary suspensor (e_1 or e_2); ×35. Fig. 13 shows four primary embryos of similar stages under greater magnification; ×72.

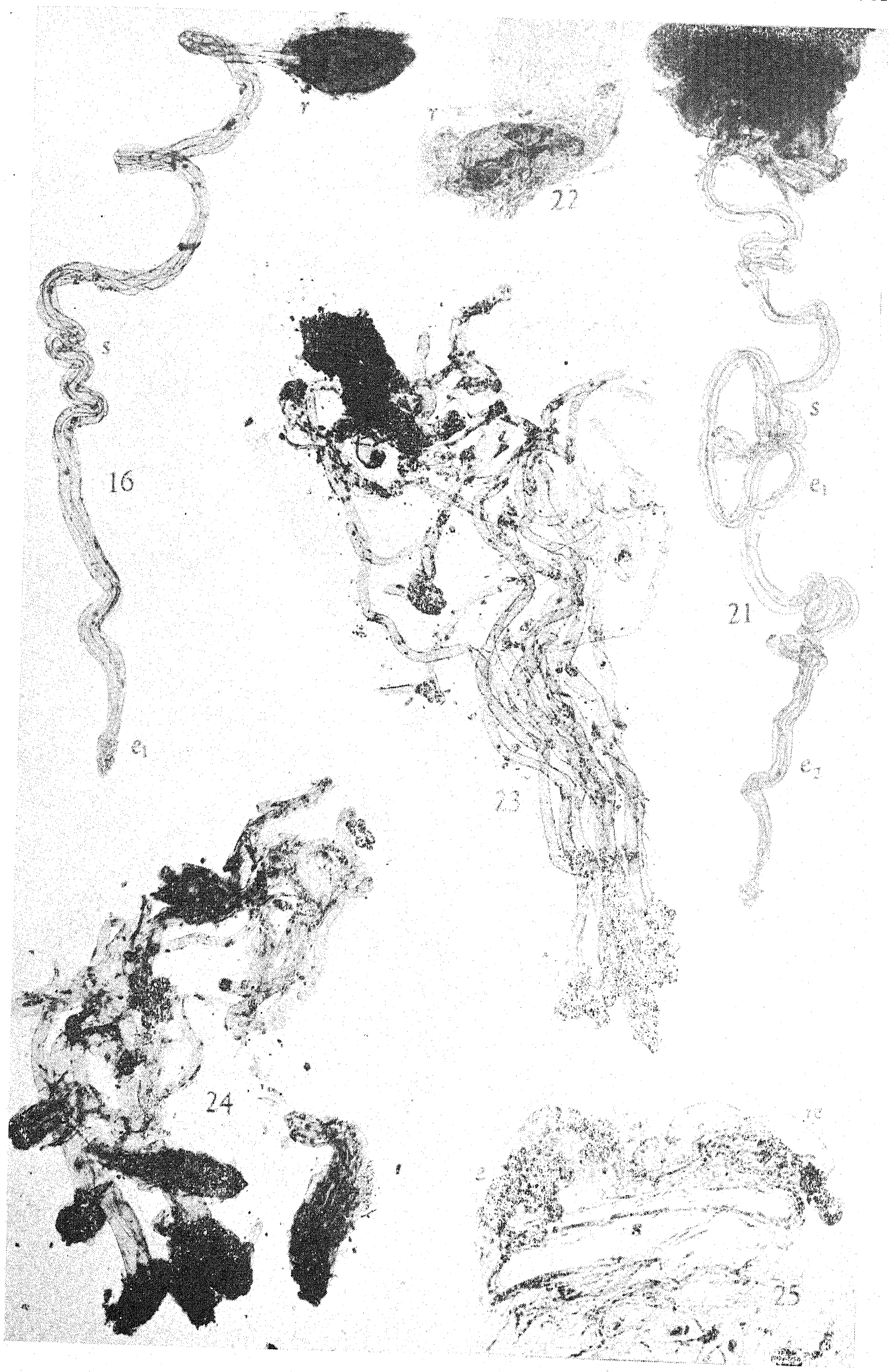
FIG. 12.—System of four embryos derived from fertilization of single egg in *Pinus Laricio*, with rosette nearly collapsed at *r;* ×35.

FIGS. 14, 15.—*Pseudotsuga taxifolia:* three of the four archegonia (probably one other archegonium lost in dissection) giving rise to three embryos resulting



BUCHHOLZ on CONIFERS

BUCHHOLZ on CONIFERS



BUCHHOLZ on CONIFERS

from fertilization of three eggs; ×35. Fig. 15, enlarged detail of upper region of suspensor cells, showing absence of rosette cells; ×105.

FIGS. 18–20.—*Cedrus libani:* figs. 18 and 19, rosette regions which have produced rosette embryos; fig. 20, single primary embryo of *C. libani* after cleavage of zygotes (see also fig. 16); ×105.

PLATE VII

FIG. 16.—*Cedrus libani:* young embryo system before cleavage of embryos, showing rosette (*r*); very long primary suspensor (*s*); and beginning of secondary suspensor (e_1); (better view of early rosette may be seen in fig. 17); ×35.

FIG. 21.—Embryo of *Picea excelsa* with primary suspensor and two sections of secondary suspensor elongated; only one embryo produced from each zygote; ×35.

FIG. 22.—Detail of rosette cells of *Picea excelsa;* rosette cells are usually collapsed as shown here; ×105.

FIG. 23.—*Libocedrus decurrens:* embryo complex of many primary embryos produced through cleavage of zygotes; ×35.

FIG. 24.—Slightly older stage than fig. 23; primary embryo at extreme left undergoing further cleavage; ×35.

FIG. 25.—Enlarged detail of rosette region of embryo shown in fig. 6; group of rosette embryos at left slightly out of focus; rosette embryos to right with elongated suspensors unusually well developed rosette embryos; ×72.

THE EL CONSUELO CYCADEOIDS

G. R. WIELAND

(WITH ONE FIGURE)

A general account of the fossil plants of the El Consuelo section of southern Oaxaca, together with detailed stratigraphic measurements, first appeared in 1913.[1] This paper was published as a preliminary to a larger memoir on the plants, later published by the Mexican survey.[2] The first mention of the great wealth of fossil plants of the El Consuelo section and surrounding region, however, was given in a short paper contributed from the Instituto Geologico shortly after the main collections were made in 1909.[3]

Simply characterized, the El Consuelo section cuts a series of mid-Mesozoic freshwater sedimentaries 560 m. thick, perhaps including some Rhaetic, the representative Lias, and the Inferior Oölite, or the Bajocien. On the Rio, or Barranca Consuelo from which the section takes its name, the sedimentaries rest on an eruptive floor and are superposed by marine rocks of mid-Jurassic (Oölitic) age. The section is the type of its kind for the North American continent, and because of the excellent and abundant series of fossil plants it bears, ranks as one of the most important well measured sections of mid-Mesozoic freshwater and marine sediments on the globe. No known region promises the student of upper Rhaetic, Liassic, and doubtless inferior Oölite plants a finer series of forms, or more hope of new discovery than the Mixteca Alta of Oaxaca, Guerrero (near Tlapa), and Puebla. Here especially the members of the Williamsonian tribe flourished in luxuriant abundance. Here grew some of the early angiosperms.

Any facts relating to the plants and the stratigraphy of the El Consuelo section are thus of the utmost interest, and it is of particular importance to record two contributions of the past ten years

[1] The Liassic flora of the Mixteca Alta of Mexico, its composition, age, and source. Amer. Jour. Sci. **36**:251–281. 1913.

[2] La Flora Liasica de la Mixteca Alta. Boletin 31, Soc. Geol. de Mexico. 1916. pp. 160. with atlas of 50 plates (by WERNER & WINTER, Francfurt o/M.).

[3] The Williamsonias of the Mixteca Alta. BOT. GAZ. **48**:427–441. 1909.

affording either new data, new viewpoints, or extensions of earlier views. One of these contributions adds somewhat to the Liassic flora, and is by ENRIQUE DIAZ LOZANO.[4] The other is a critique (with new facts), the title of which is partly explanatory, by BURCKHARDT, so well known for his contributions to the paleontology and geology of Mexico.[5]

I. Cycadeoids of Huayacocotla and Huachinango

It will be convenient to consider first the fossil plants of Lozano. These come from what are apparently two localities in extensions or rather isolated outliers of the Liassic of the El Consuelo section. The first is near the village of Huayacocotla, in the state of Vera Cruz, on the mountain front facing the Gulf at an altitude of 2200 m., in 20°30′ N.L. At the mountain foot the Rio Viñasco, a branch of the Tuxpan, cuts a deep ravine into the frontal escarpment, revealing lower Jurassic and superposed beds. The second plant locality is in Puebla, extending along the Barranca traversed by the Rio Necaxa, where some 4 sq. km. of the plant beds appear to view near the village of Huachinango.

As collected so far, the plants from these two new stations are rather fragmentary, and do not include such fine type specimens as those of the El Consuelo and neighboring sections; but the new localities nevertheless add very interesting forms to the flora already known. As described by LOZANO, the specimens include larger fronds of the *Otozamites Molinianus* Zigno type, a well marked Podozamite of a very fine venation, *Ptilophyllum*, so prominent in the middle strata of the El Consuelo section, two fine specimens of *Pterophyllum*, and a *Cycas* or *Cycadites*. Although mostly single pinnules or but fragmentary portions of fronds, these are really valued additions to the previous series.

A critique of the LOZANO plants is more intelligible if the reader has access to the illustrations, which are clear enough when compared with the plates of the Flora Liasica. The new plants then

[4] Unas Plantas Liasicas de Huayacocotla (Vera Cruz) y algunas Plantas de la Flora Liasica de Huachinango (Puebla). Bol. del Instituto Geológico de Mexico, no. 34. pp. 18. *pls. 9.* 1916.

[5] Quelques Remarques Critiques sur l'ouvrage de M. W. FREUDENBERG "Geologie von Mexico," par Dr. C. BURCKHARDT. Mem. Soc. Cientifica "Antonio Alzate" **41**:185–196. 1923.

seem to be an extension of the plants more definitely of Liassic age, as LOZANO concluded. It may be noted that the *Pterophyllum* is perhaps of persistent type. A lesser species of fine venation (1 cm. = 12 v) is close to the *P. propinquum* (cf. SCHENK, Keuper und Lias Frankens), and also easily compared with Liassic species of the Rajmahals (cf. FEISTMANTEL, Jurassic-Liassic Flora of the Rajmahal Group, p. 58). Another *Pterophyllum*, of the Huyacocotla group, represented only by numerous fragments it is true, seems correctly approximated by LOZANO to larger Indian types. It is comparable with the *P. princeps* of OLDHAM and MORRIS; while transition from the fine to the larger and coarse-veined pinnule type is exemplified by the Rajmahal *P. Carterianum*. In passing, one remarks that there were many and striking cosmopolitan elements in the floras of Liassic time, but as in modern types area (extent of distribution) tends to indicate age, so even in the case of these world-wide types of the Liassic, the ever present probability is long persistence in time. The *Ptilophyllum acutifolium* is conspicuously present in the series (cf. LOZANO, Laminas IV–VI, most figures).

In the Lias of Oaxaca no foliage referable to *Pseudocycas* or *Cycas* was found, although one poorly conserved carpellary series was seen and illustrated. Certainly the seeds were present, and the appearance of the matrix suggested the presence of a carpellary crown (cf. pl. 37 of the Flora Liasica). As the Cycadaceae are merely Eastern World relicts, the foliage type has little stratigraphic value, and it is of only casual interest to find in the LOZANO plants a single example. This is, however, quite in error viewed as a species of *Otozamites*, for inspection of the figure (*loc. cit.* pl. 4. fig. 2) suggests elongate insertion of the pinnules, instead of the eared condition doubtfully noted. The appearance is that of slightly overlapping pinnules with a single midrib. LOZANO, in outlining the pinnules of one side of the rachis, seems to have been misled by the presence of a midrib, and so failed to note the inconsistent appearance of his figure. For if this is not a *Cycas* with a single midrib and absence of other venation, there would be some six broader pinnules on the one side of the rachis, corresponding with ten or twelve narrow pinnules on the opposed insertion line. Such an inequality is not possible. Besides, the six broad pinnules show traces of the midrib or vein, and

have the double breadth nearly enough. Again, if the outlining were correct, there would be the curious condition of the displacement of the narrower pinnules in pairs. Until better material is secured, this plant may be regarded as a *Cycadites* of indeterminate species, with fronds under 1 m. in length and 12 cm. or more across, the pinnules being a scant centimeter broad.

Several true species of *Otozamites* are present in the Lozano series, however, as shown on Lamina III, and these are of the greater interest because closely related to the *O. hespera* from stratum 19 in the lower part of the El Consuelo section. One of the forms has a rather broad pinnule, recalling somewhat the *O. Molinianus* Zigno of the Italian Lias. The slight difference between the latter and *O. hespera* is about accounted for by the new pinnule forms. It may then be said that *O. hespera*, taken with two nearly related but distinct species not as yet conveniently named, forms a compact trio of marked Liassic stamp, while the near relationship of this trio of species to certain types of the European Liassic is emphasized.

As showing the difficulties faced by the paleophytologist, the group just cited, with inclusion of the *O. Molinianus*, further relates itself to the *O. obtusus* of Lindley and Hutton from the Liassic of Axminster; and then in turn the general type runs up into the Inferior Oölite *O. graphicus*. In fact, *O. obtusus* has perhaps gained a rather comprehensive status as a species of Liassic-Oölitic range. The form called *O. obtusus* var. *oöliticus* (Seward, Jurassic plants, Pt. I, pl. I) does however appear distinct.

An *Otozamites* (Lozano, Lamina IX) referred to the *O. Hennocquei* is not distinctive, relating itself to both Liassic and Rhaetic types. Similarly another form (Lozano, Lamina I, fig. 1) is like the Rhaetic *Podozamites distans*.

Andriania.—Before taking up the notes of Burckhardt, it is desirable to make a slight correction of my determination of the oldest plant found in the El Consuelo section. This fossil is from the beds about 35 m. above the base, and thus from well within the strata which could be of Rhaetic if not of Liassic age. It is a portion of a large and fine frond, well outlined, but of indistinct venation, figured as a possible *Laccopteris*.[6] When the description was given,

[6] La Flora Liasica, Lamina XLIV, photograph 3.

however, the features of the *Andriania* of BRAUN, doubtless with *Laccopteris* a member of the Matoninae, had not been noted. Lately a paper by GOTHAN[7] came to hand with an account of forms of *Andriania* of Rhaetic age, which at once recalled the supposed El Consuelo *Laccopteris*, for in *Andriania* there is a very peculiar and characteristic combination of form and venation which, once well noted, would never again be overlooked. Medium sized species have fronds about 8–12 cm. across, with narrow basally confluent pinnules about 6 mm. broad. To this characteristic form of frond is united a peculiar areal, rather than lobate venation, the vein areas being very small, only about 1 mm. broad by 2.5 mm. long. Into each of the succession of these small oblong vein areas, in the species *A. norimbergica*, the strong midrib of the pinnule first sends off a vein to the outer border, with also an anterior short branch vein to the border, and then more basally a longer fork to the border, followed by a single low fork from the midrib. Whenever this simple order of the veins repeats itself, there is each time produced a furrow due to the long vein, and a roll or ridge due to the forked veins, such as is rarely seen in any other fern (fig. 1). In the El Consuelo frond the areal feature is present, although the finer venation cannot be seen. Nevertheless the proportions and all visible features are those of *Andriania*. The first specimen found with clear venation will doubtless prove the point.[8]

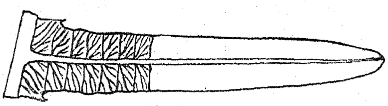


FIG. 1.—*Andriania norimbergica* (Rhaetic of Nürnberg), after GOTHAN.—Vein areas 9 or 10 to the cm. and pinnule width 5 mm., in agreement with *Andriania* (*Laccoperis* ?) sp. of base of El Consuelo section.

Representatives of the Matoninae have not seemed abundant in the Western World so far; but I may add that I noted, in the collections in the Instituto Geologico from the Sonoran Trias, fertile fronds with features so distinct that they could be referred to the Old World species *Laccopteris Münsteri* without question. In speak-

[7] Abh. d. Naturhist. Gesells. Nürnberg. XIX. 14:pl. 17.

[8] The manner in which *Andriania* relates itself to *Laccopteris* in both fruiting and venation is illustrated by another curious Rhaetic fern from Skone recently described by JOHANSSON under a new generic name *Pterygopteris*. This fern also suggests transition toward *Dictophyllum*. See JOHANNSON, Arkiv för Botanik, K. Svensk Vet-Ak. 17: no. 16.

ing of those greater trends of change and migration which in later Mesozoic times cut off many of the Matoninae, and which have restricted the sole remaining representatives to a southern rather than to the northern region they once occupied, SEWARD is much impressed by the long persistence of *Gleichenia*, *Dipteris*, and *Matonia*. He states:

> Whatever may have been the main trend of migration, and wherever the ancestral homes may have been, such ferns as *Gleichenia*, *Matonia*, and *Dipteris* are impressive examples of constancy in a changing world; in the course of their wanderings from one part of the earth to another they have suffered but little change since the days which preceded the mysterious rise to ascendency of the flowering plants.

The clearer, the more pointed, and direct it is to add that the representatives of the Matoninae can have, taken alone, but little stratigraphic value after their first known occurrence about Rhaetic time. Even then none may know whether the type was quick or slow to evolve; although once evolved such a type could reach a more or less cosmopolitan distribution almost instantaneously in the lapse of geologic time. Persistence of *Andriania* in the Wealden of France is recorded by CARPENTIER.[9]

The probable occurrence of a typical *Andriania* in the basal strata of the El Consuelo section, however, suggests a certain accord with the observation of BONILLAS, then of the Instituto Geologico, that heavy beds in the valley of the Nochixtlan southerly from Tlaxiaco, carry many Taeniopterids, recalling the Sonoran Trias with its great masses of *Taeniopteris*, and with *Laccopteris*, and many other striking plants.

II. Where does the Lias of the El Consuelo section end?

The El Consuelo section was called the type Liassic section for North America because it was believed to cover Liassic time, with the Rhaetic and Oölite affording the extreme boundaries. Resting on an eruptive floor, the freshwater sediments, nearly 600 m. higher up, run under marine horizons, which in the judgment of BÖSE and

[9] Sur les Conifères et les Fougères du Wealden de Fèron-Glageon (Nord). Ext. Compt. Rendu. Acad. Sci. 1922 (pl. 174).

of BURCKHARDT are middle Oölite. The prominent features of the section follow:

1. Entire thickness of plant beds is nearly.................. 600 m.
2. From eruptive floor to *Andriania*, the oldest plant found in the section.. 45 m.
3. From *Andriania* to the *Williamsonia Nathorstii* casts...... 40 m.
4. From eruptive floor to principal coal seam with *Cordaites*, *Noeggerathiopsis*, and *Otozamites Mandelslohi*, etc......... 100 m.
5. From main coal to *Otozamites hespera* and *Araucarioxylon* log.. 140 m.
6. Approximate thickness of lower plant beds............... 250 m.
7. Main coal to main *Williamsonia* horizon................ 250 m.
8. From *Williamsonia* with large foliage types to highest point at which distinct plant species were found, about........ 150 m.
9. From horizon 61 and last of the plants to the marine middle Oölite.. 85 m.
10. Base to main *Williamsonia* horizon.................... 350 m.

First suggested division:

From eruptive floor to above *Andriania*, Rhaetic....... 80 m.
Williamsonia Nathorstii to horizon 61, Liassic.......... 400 m.
Horizon 61 to marine beds (mid-Oölite), Bajocien...... 85 m.

Second suggested division:

From eruptive floor through stratum no. 41, Liassic.... 440 m.
Beginning with horizon 42, where the megaphyllous *Williamsonia* types are first conspicuous and a certain resemblance to the Oölite plants of the Yorkshire coast is first evident, and then extending through horizon 61 to the marine beds, Bajocien.......................... 130 m.

Third suggested division:

Lower beds (with most of the coal), Lias.............. 250 m.
Upper beds, beginning above stratum 19, and extending through uppermost strata for 85 m. without distinct plants, Bajocien.................................. 320 m.

Of these suggested divisions the first appears the most probable. A certain emphasis must also be laid on the volume of the sediments composing the El Consuelo section in making broader comparisons with the British Jurassic. The ratio of the maximal thickness in these far separated regions must have some relation; in fact, a fairly direct relation to time division. It may be laid down as law that only from all the fossil evidence, and only from the greater sections, or else those very definitely correlated with one another,

can it at some later time be affirmed that any plants of given strata of the Yorkshire coast lived at the same time as certain given plants from the freshwater beds of Oaxaca, or of Puebla, or of Guerrero. The El Consuelo section is probably the most massive lower Jurassic section to be found in Mexico, but the outliers are either unstudied or afford but very partial comparison so far as known. The extent of the beds and the striking outlines of the vegetation they contain make further investigation in field and laboratory extremely urgent. It is convenient to quote from GEIKIE:

> The Jurassic formations stretch across England in a varying band from the mouth of the Tees to the coast of Dorsetshire. They consist of sands, sandstones, and limestones interstratified with softer clays and shales. Hence they give rise to a characteristic type of scenery, the more durable and the more porous beds standing out as long ridges, sometimes even with low cliffs, while the clays underlie the level spaces between.

The contrast between the English Jurassic landscapes and those of the Mixteca Alta is indeed great.[10]

But from the extent of the beds in both of these great type regions, the sequence in which they occur, and the similarity of important floral elements, it is certain that coordinated study and

[10] The ten or twelve thousand feet of Mesozoic strata which give character to the Mixteca Alta are about equally divided between the Jura and Cretaceous, any Trias that may occur not being as yet separated or defined. The Mesozoic mass is often entirely freed from Tertiary eruptives, ash, or conglomerates, and rests on older sedimentaries of undetermined age, or against intrusives. The topography is exceedingly rough. The streams, low in the dry winter and torrential in the rainy midsummer, in cutting through the massive Cretaceous limestones capping Jurassic strata of lesser induration, form a splendid system of flood-cut valleys, gorges, and canyons. Yet because of the more varied vegetation and rather free growth of pine and oak with relatively few barren stretches, scenic aspects are far softer than in the upland mountain country of central Mexico. Subjected as this region has long been to tremendous tectonics, the marine and underlying freshwater deposits form no long crests. And especially the fresh or brackwater sediments 2000 feet thick as deposited along the south continental border for the lower half of Jurassic time, along with thinner marine wedges, are now found where somewhat protected from the full effects of dissection and erosion by border or transverse ranges. Extended areas of the Lias and inferior Oölite are not seen, although in the limited and isolated areas where the rocks of these periods form the surface topography, interesting changes in the vegetation occur. Thus along the Barranca Consuelo the scattering oaks of the eruptive floor contrast sharply with the pines and more varied growth of the Liassic slopes.

Of these isolated Liassic-Oölitic areas extending up to the time when subsidence became pronounced, and all the south continental region was finally invaded by the sea

comparison is but begun. The freshwater divisions of the El Consuelo section correspond in larger outline to the basal development of the British Jurassic rocks. It is therefore of first interest to note the great preponderance of Liassic strata in the British maxima, as follows:

Bajocien=	(*a*) Cheltenham beds or thick estuarines (Inferior Oölite) of Yorkshire up to "Cornbrash".......	82 m.	= 94 m.
	(*b*) Northampton sands ("Dogger" of Yorkshire), and		
	(*c*) Midford sands (passage beds)............	12 m.	
Lias	Upper Lias..............................	21– 62 m.	=459 m.
	Middle Lias..............................	18–105 m.	
	Lower Lias..............................	148–292 m.	
Bajocien+Lias, maximum........................		553 m.	

It is thus seen that the El Consuelo section, one of the most accurately and satisfactorily measured in the world so far as the plant beds go, measures almost the same as the British section (570 to 553 m.); while the parallel even extends to the ratio of Liassic to Bajocien sediments, if my first suggested division holds. Had these suggested or trial divisions of the El Consuelo section been made when the Flora Liasica was published, by either the author of that work or anyone else, on the basis of the facts then available, the results reached would have been about the same. Were not the main facts very evident? Was it not emphasized that the great thickness of the section and the many successive phases of sedimentation suggested that one or several unconformities might lie hidden in that great thickness of sediments? Was it not shown that a typical Liassic flora was present, one that looked old enough to extend to the base

about mid-Oölitic time, a number are now known. Of these may be mentioned (1) the Tlaxiaco River valley; (2) three to five kilometers northwesterly from the town of Tlaxiaco; (3) at Mixtepec on the Rio Mixtepec; (4) east of the Cerro del Lucero in the Tezoatlan-Rosario region, a most important unstudied locality; (5) all of the Barranca Consuelo as yet most imperfectly examined for its great store of plants; (6) at the "Pina de Ayuquila" near the village of Ayuquililla in the state of Puebla; (7) near Tlapa in the state of Guerrero, a fruit locality; (8) Huchinango, Puebla; (9) Huayacocotla, Vera Cruz; and (10) the important unstudied region of the valley of the Rio Nochixtlan to the south of Tlaxiaco. The plants yet to be collected in the Mixteca Alta must make those already secured seem small and insignificant in both preservation and number. This region is one of the most favored on the globe for the study of the plant life of the entire Liassic formation, and probably most or all of the Inferior Oölite time.

of the Lias? Was it not seen that some Rhaetic strata might be present? That there was an older phase in the flora? That there were typical *Williamsonia* cones of the largest size among the plants far down toward the base of the section; and that *Williamsonia* became most abundant and varied with ascent in the beds to the point above which, with changes in the character of the sediments, no more plants were found? Was it not seen that the beds of the upper part of the section which did not chance to yield plants were 85 m. in thickness, and might thus easily cover the gap in sedimentation between the close of Liassic time and the superposed marine strata of middle Oölite age; that is, represent the Inferior Oölite, the Bajocien?

It was not for a moment to be inferred that these strata above horizon 61 belonged to the Lias. There was no evidence from the plants, and the question became largely geologic because the flora of the section could be seen to have changed with ascent in the section to near, if not actually into, a true Inferior Oölite plant facies. It was obvious that evidence from other sections, both plant and stratigraphic, ought to be secured before attempting to define the upper limit of the Lias. It was understood earlier, and the fact may be emphasized now, that the transition from the *Williamsonia* elements of the Lias (the chief plants concerned) into those of the Inferior Oölite is not sharp but gradual, and on last analysis undefined. It could easily be that the large leaved *Williamsonia* forms beginning about horizon 42 must finally be referred to the Bajocien.

The point was that the plant collections from the El Consuelo section extended the geographic range of the lower Jurassic floras so notably that it seemed the more satisfactory, even the more philosophic, to let this new body of evidence thus stand out by itself. The outstanding uncertainty was of the upper boundary of the Lias. It was thought, however, that any attempt at a discussion must be more or less premature, especially as more work in the field was then and is still the great need. It is well sometimes to recall the somewhat taunting remark of HUXLEY when it comes to synchronizing far separated sedimentaries and the ancient floras they bear:

> Geologists have imagined that they could tell us what was going on at all parts of the earth's surface during a given epoch; they have talked of this deposit

being contemporaneous with that deposit, until, from our little local histories of the changes at limited spots of the earth's surface, they have constructed a universal history of the globe as full of wonders and portents as any other story of antiquity.

Attention may be called, however, to this early statement of opinion as to the age of the lower Jurassic plants of the Mixteca region (cf. La Flora Liasica de la Mixteca Alta, p. 154, where the words are italicized): "Por lo tanto se deduce que las capas de plantas del corte de El Consuleo están en el límite superior del Rhético y probablemente se extienden al Liásico, cerca del Oölítico inferior." The notes in English read: "It is therefore found that the plant beds of the El Consuelo section begin at the upper borders of the Rhaetic and probably extend through the Liassic near to the lowermost Inferior Oölite." This should refer to the plant beds in the narrower sense of those yielding well defined plant species. Strictly taken the statement could not refer to the basal barren strata; and it emphatically could not refer to the beds above stratum no. 61, now accepted as clearly supra-Liassic.

Of course, in speaking of "plant beds" without reference to age, all the sedimentaries were included as a convenience, from the base of eruptives to the final invasion of the sea in mid-Oölite time, but the fact was repeatedly emphasized that the lower limit of the Lias, and much less the upper limits, could not at once be defined. It was pointed out "that it was not believed that the history of the plant beds consisted in a single unit," and that while no unconformities were found, "several might be present." The opinion was also ventured (and is held still) "that the plants of the Inferior Oölite of the Yorkshire coast are really a left-over Liassic, rather than a typical mid-Jurassic facies." These were mostly the cosmopolitan types that might readily have existed during the deposition of the underlying "Midford sands." These latter contained at their top the famous Gloucestershire "Cephalopoda beds," did not yield fossil plants, were recognized as transition beds, and sometimes actually assigned to the Lias.

From all that has been said about the status of opinion as to the age of the rocks of the El Consuelo section when first measured fifteen years ago, it can be understood how welcome is the finding in

the Oaxacan region of certain marine wedges bearing more or less distinctive Ammonite types tending to throw light on the position of the upper limits of the Liassic. Those interested must turn to the papers of BURCKHARDT for mention of this evidence (1913 and 1923). He states that the freshwater beds of Oaxaca–Puebla must have been deposited along the borders of a Continent (as was earlier granted), since there occur marine interpolations bearing Bajocien *Ammonites*, and the series is in turn overlain by the Bathonian (at Cualac, Guerrero), and by the Callovian in the higher or marine portion of the El Consuelo section. Both formations are rich in *Ammonites*. BURCKHARDT says more recently (1923) of the freshwater series of the El Consuelo section, that my original tentative division into lower and upper plant beds is near a true division into Lias and Inferior Oölite (Bajocien):

> La partie inférieure de la série de couches avec plantes et charbon d'Oaxaca doit être liasique d'accord avec les résultats de Wieland, qui a fait resortir les affinités liasiques de la flore d'Oaxaca tout en méconnaissant l'importance stratigraphique d'autre éléments de la flora qui montrent des rapports intimes avec les plantes oölithiques de la côte du Yorkshire.

That is to say, if I understand BURCKHARDT precisely, he would include in the Lias about the lower 250 m. of the freshwater sedimentaries of the El Consuelo section, or the part containing most of the coal, and in the Inferior Oölite (Bajocien) the upper 320 m.

This division, based upon a much needed further study in the Mixteca Alta region, stratigraphic as well as paleobotanical, may prove to be essentially correct. It is also contended that, taken as a final criticism, such a result would not lie outside the views already outlined at all. The flora offers no obstacle. Above horizon 41 (*cf.* supra) the recovered flora fairly agrees with the Inferior Oölite plants of Yorkshire, certain distinctive species merely appearing a little more megaphyllous, a little more tropical.

There are, nevertheless, in Oaxaca, Puebla, and Guerrero, a number of great sections bearing abundant plants far better conserved than those described by LOZANO from Huachinango and Huayacocotla as already commented upon, which must primarily be brought into exact comparison with the El Consuelo section, before the larger relationships and fuller stratigraphic significance of the

luxuriant and varied cycadeoid floras of the Mixtecan region can be well enough understood. Is it not the outlining of a great field of future study rather than mere technical discussion that is so impressive in this further passage from BURCKHARDT'S critique on FREUDENBURG'S *Geologie von Mexico?*

Dans le chapitre sur le Lias on trouve une reproduction de la coupe de la Barranca de Huayacocotla de E. BOESE. Les observations que j'ai pu faire en 1913 dans la region de Huayacocotla et Huachinango m'ont demontré que le profil cité de BOESE est inexact. En effet le terrain liasiques, au lieu de se présenter sous forme d'une série de schistes uniformement inclinés avec intercalation d'un grés au milieu, se compose d'une dizaine de zones paléontologiques qui sont très fossilifères et réprésentent une série du Lias inférieur et moyen depuis le niveau de l'*Arietites Bucklandi*, jusqu'à celui du *Polymorphites Jamesoni*. Une zone riche en Bivalves (nombreux *Pecten*, *Trigonia*, et.) contient des roches gréseuses et se présente vers le milieu de la série, au dessus d'un niveau à *Oxynoticeras*. Un peu plus haut s'observent des couches rempliés d'*Echinoceras* du gr. *raricostatum*. Des schistes et grés avec plantes terrestres, dont plusieurs seraient identiques avec des plantes d'Oaxaca, étudiées par WIELAND, et qui ont fait l'objet d'une étude de E. DIAZ LOZANO, sont surmontés par une série transgressive du Jurassique supérieur débutant par un conglomérat de base. Toute cette série est fortement plisée et morcellée par de nombreuses failles.

The fact should be emphasized, therefore, that the several contributions bearing on the age of the plant beds of Oaxaca and Puebla merely form initial and as yet little coordinated studies of this remarkable region. Each successive observer has had to depend primarily on such data as could be assembled during a rapid reconnaissance. That this condition might arise was indeed early noted regretfully by both BÖSE and BURCKHARDT. The responsibility for one failure to continue study and collection in the Mixteca Alta rests on me. AGUILERA, then director of the Instituto Geologico, suggested early in 1910 that it might be better to resume the work in the south, instead of going to Sonora. Had I in the least suspected that fifteen years would pass without further work on the El Consuelo and neighboring sections, I should not then have given precedence to other work in new fields. As events fell, this is the first time that I have been able to give anything like due attention to the data brought to light by others.

It may also be added that it is quite possible that the interesting cycadeoid foliage of the Tlaxiaco River locality (particularly the

fine species *Otozamites paratypus*, Flora Liasica, Lamina XVI, photograph 8), belongs higher in the general sedimentary series than I was at first led to suppose. In that case the large leaved El Consuelo *Williamsonia* forms would stand more isolated as the new species coming in sharply with the advent of Bajocien time; although it was held and emphatically stated that the vegetation of the higher strata of the section appeared juvenile. Such species as *Otozamites paratypus*, *Oaxacensis*, *Aguilariana*, *Diazii*, and *Aguilerii* had led to the idea of newness, or recent and local origin. Indeed these plants, taken as a tropic phase of the greater cycadeoid facies, might well have lived when the Midford sands were laid down. If so, not merely will their evolutionary interest prove marked, but their stratigraphic value will become tangible.

I need not refrain from remarking again that the "stratigraphic significance" of the extinct plants or animals is an intrinsic thing depending on demonstration from available data. It does not rest on surmise, and it seems to me that geologists are apt to expect too much of the plant record, as yet little coordinated, and so often consisting in the indeterminate types of regions remote from each other. Thus the striking *Williamsonia* fruits of the Inferior Oölite, although deeply interesting in discussions of the origin of the angiosperms, are of a more limited, and even local stratigraphic value than is commonly inferred. The cycadeoids cannot have a value like that of *Ammonites* in time determinations. In the case of the dissociated stems, fruits, or leaves of such conservative plant types identification of species is difficult; but in that of the Ammonite shell, which is the full index of a complete organism, identification is easy. It must be admitted that the fine El Consuelo series of *Williamsonia* cones does not disclose more than those broader specific relationships to the cone series of the Yorkshire coast. The features used in specific determination of isolated cones are variable rather than exact. There are no successive series affording certainty of the course of cone persistence and change as yet visible. *Williamsonia Wettsteini* of the Keuper of Lower Austria, the oldest cycadeoid cone thus far discovered, is a large seeded type, which may easily run back to the base of the Trias. The *Cordaites* of the El Consuelo section proved an unexpected persistence of that group; and con-

versely let any one say with caution that any particular cycadeoid species or group of species is young, new, and marks the beginning of Inferior Oölite time.

Conclusions

The several plant-bearing strata of the Lias and Bajocien of Mexico, especially those of the localities and sections herein enumerated, mark one of the most favorable regions of the globe in which to search for antecedent and primitive angiosperms. Accessibility and climate are both very favorable. Conservation of the finest details of leaves and fruits is frequent. Either as imprints or as carbonized forms, many of the types must prove remarkably satisfactory objects for study by the exacter chemical methods. The new angiosperm group Caytoniales, as recently demonstrated from the Inferior Oölite of the Yorkshire coast by THOMAS, is certainly present in the lower strata of the El Consuelo section, where the fine leaf type *Sagenopteris* (?) *mexicana* is barely distinguishable from *Sagenopteris Phillipsi*. That is, this angiospermous foliage positively extends to the base of the Jura in Mexico. Moreover, in the higher or Bajocien strata there is another type of foliage, at first referred tentatively to the Glossopterids, but possibly angiospermous. This occurs as elongate blades inadequately illustrated in the Flora Liasica (Lamina XLVI, photograph 3) as *Glossopteris* (?) *mexicana*. Form and venation are just what might be expected in the Jurassic forerunners of the Lower Cretaceous dicotyls.

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CHANGES IN NITROGEN, POTASSIUM, AND PHOSPHORUS CONTENT OF WHEAT SEEDLINGS DURING GERMINATION AND EARLY STAGES

JEHIEL DAVIDSON

In plant nutrition studies, the absorption of food elements is estimated either by analyzing the residual medium, the difference between it and the original medium giving the quantities absorbed by the plants, or by directly analyzing the plants. The first procedure can be used conveniently only in the case of water cultures. The second procedure is of general application, and would seem to be preferable, being more direct. In analyzing the plants directly, whenever the growth of controls is not feasible, investigators usually compare the quantities of food elements in the seedlings or plants with those in a corresponding number of seeds from the same stock which was used to prepare the seedlings. The fact that changes may occur in the seeds during the process of germination is generally overlooked. These changes may be caused by leaching into the medium or by absorption from ungerminated seed leachings and from tap water. Moreover, seedlings come from vital seeds only, but most stock seed contains some that are sterile, and these may deviate in composition from normal seeds.

This investigation was undertaken to determine experimentally whether seedlings, at the ages at which they are generally transferred to water or sand cultures, differ in composition from the stock seed from which they originate. Should it be shown that under normal conditions appreciable changes take place in the seeds during germination, the composition of plants at the termination of experiments will have to be compared with the composition of the seedlings which were used to start the experiments, instead of with that of the stock seed. The question, besides its bearing on experimental methods, is also of general interest.

Changes in composition of seedlings and ungerminated seeds during germination in tap water

In the Crop Chemistry Laboratory, seedlings are grown from seeds carefully spread out on perforated aluminum disks floating on

tap water, so that the individual seeds will not come in contact with one another. This experiment was conducted in two series: the "sparse," in which wheat seeds were spread out for germination in the usual way; and the "dense," in which the seeds were spread in a continuous layer, each seed being in close contact with the surrounding seeds. A purple straw soft winter wheat of the crop of 1924 was used. At intervals of five and seven days, counting from the time the seed was set to germinate, seedlings, as well as seeds which failed to germinate, were removed and analyzed. All the ungerminated seeds were not necessarily naturally sterile; some seeds, especially in the dense series, probably failed to germinate for some cause other than sterility. A complete set of nitrogen determinations was obtained; phosphorus and potassium determinations were made only on the seeds and seedlings removed from the thickly planted aluminum plate after the seven day period. Aliquots of 30 seeds or seedlings were used for the various determinations, which were made in five replications, except the nitrogen determination in the stock seed, which was made in ten replications. The average results are given in tables I and II.

The nitrogen figures (table I) show that the nitrogen content was appreciably affected by the way the seeds were spread on the aluminum disks. The losses from the ungerminated seeds were not very consistent. Those which were removed after five days lost appreciably in the dense series, but showed no loss in the sparse series. After seven days the ungerminated seeds sustained losses in nitrogen in both series, the losses being appreciably larger in the sparse series. It has been shown elsewhere[1] that no nitrogen in gaseous form is lost during germination. The recorded losses of nitrogen have evidently occurred through leaching. The apparent reversal of conditions with reference to nitrogen losses from ungerminated seeds in the five and seven day sets may be merely accidental, or may be explained by assuming that two factors are involved in the solubility of nitrogenous material in seeds, the state of decomposition of this material, and the solvent action of the medium. Decomposition was probably going on more actively in the dense series, as conditions there were more favorable to microbiological activity than in the sparse series.

[1] DAVIDSON, JEHIEL, Is gaseous nitrogen a product of seedling metabolism? BOT. GAZ. 86:95–101. 1923.

On the other hand, owing to the far greater abundance of ungerminated seeds, the dense series would be more likely to be influenced by the limited solvent action of the medium than the other series. This explanation would seem to be borne out by the fact that the losses from ungerminated seeds in the dense series did not increase after the five day period. While the total loss from the ungerminated seeds after seven days might have been greater in the dense series, the individual losses from seeds were greater in the sparse series.

TABLE I

CHANGES IN NITROGEN CONTENT* IN SEEDLINGS AND UNGERMINATED SEEDS DURING GERMINATION IN TAP WATER

Period	Stock seed	Ungerminated seed (mg.)		Seedlings (mg.)	
		Dense	Sparse	Dense	Sparse
....	20.82	....	....	....	....
5 days..................	....	19.78	21.07	21.42	21.07
7 days..................	....	20.06	19.12	22.76	21.40

* Aliquots of 30 seeds or seedlings.

TABLE II

CHANGES IN POTASSIUM AND PHOSPHORUS CONTENT IN SEEDLINGS AND UNGERMINATED SEEDS DURING SEVEN DAYS' GERMINATION IN TAP WATER*

K_2O† (mg.)			P_2O_5† (mg.)		
Stock seed	Ungerminated seeds	Seedlings	Stock seed	Ungerminated seeds	Seedlings
4.3	....	....	9.9	....	....
....	2.8	5.5	....	8.8	9.4

* Dense series. † Aliquots of 30 seeds or seedlings.

The gains in the nitrogen content of the seedlings are in full accord with this explanation. After five days the seedlings of the dense series showed a gain in nitrogen as compared with the stock seed, and the nitrogen content of the seedlings in the sparse series remained unchanged. After seven days the seedlings in both series showed gains in nitrogen, but the dense series was far ahead, the nitrogen content of the seedlings being about 10 per cent higher than that of the stock seed.

The results in table II show that after seven days the ungerminated seeds sustained appreciable losses of both potassium and phos-

phorus, while the seedlings made a pronounced gain in potassium and showed a slight loss of phosphorus. This is similar in tendency to the results of LeClerc and Breazeale,[2] who grew wheat seedlings on aluminum plates without separating them from the ungerminated seeds during the entire duration of their experiments. Evidently the seedlings were not able to utilize the phosphorus which leached out from the ungerminated seeds, the process of growth protecting them only against excessive losses of phosphorus. The gains of potassium and nitrogen might have come to a small extent from the tap water, which generally contains 1.5–2 parts per million of potassium, and 0.2–1 part per million of nitrogen as nitrate.

Changes in composition of seedlings and ungerminated seeds during germination in distilled water

The experiment was repeated, using distilled water instead of tap water to germinate the seedlings. The plants did not make as good a stand as in the previous experiment, their root systems being especially inferior. The two experiments differ not only with reference to the composition and reaction[3] of the media in which the seedlings were germinated, but also with reference to the vigor of the seedlings. Hence they are not strictly comparable, and only represent a study of the same question under two sets of conditions.

The changes in the potassium and phosphorus content of the seedlings and ungerminated seeds were studied more extensively than in the previous experiment. Determinations were made in both dense and sparse series, and in sets representing three time periods. Owing to shortage of material, aliquots of only fifteen seedlings or ungerminated seeds were used for each determination, and the number of replicate determinations in the five and seven day sets was reduced to two for phosphorus and to three for potassium. For the rest of the determinations five replications were used, as in the previous experiment (average results given in tables III and IV).

The nitrogen losses (table III) from the ungerminated seeds were quantitatively about the same as those in the previous experiment, but they were more uniform and did not vary in the same order.

[2] LeClerc, J. A., and Breazeale, J. F., Translocation of plant food and elaboration of organic plant material in wheat seedlings. U.S. Dept. Agric., Bur. Chem. Bull. 138.

[3] The tap water used generally has a P_H value of 7.4; distilled water a P_H value of about 5.4.

After five days the seedlings showed slight losses of nitrogen in both series. After the seven day period, however, they showed the same gains as in the previous experiment. The seedlings of the dense series again showed an increase of 10 per cent of nitrogen over the stock seed. The deviations from the results of the previous experiment were probably due largely to less vigorous absorption.

TABLE III

CHANGES IN NITROGEN CONTENT* IN SEEDLINGS AND UNGERMINATED SEEDS DURING GERMINATION IN DISTILLED WATER

Period	Stock seed	Ungerminated seeds (mg.)		Seedlings (mg.)	
		Dense	Sparse	Dense	Sparse
....	10.41	....	....	....	....
5 days..........	....	9.97	9.92	10.01	9.75
7 days..........	....	9.75	10.09	11.42	10.70

* Aliquots of 15 seeds or seedlings.

TABLE IV

CHANGES IN POTASSIUM AND PHOSPHORUS CONTENT IN SEEDLINGS AND UNGERMINATED SEEDS DURING GERMINATION IN DISTILLED WATER

Period	K_2O* (mg.)					P_2O_5 (mg.)				
	Stock seed	Ungerminated seeds		Seedlings		Stock seed	Ungerminated Seeds		Seedlings	
		Dense	Sparse	Dense	Sparse		Dense	Sparse	Dense	Sparse
....	2.15	......	......	......	......	4.95	......	......	......	......
5 days....	......	1.45	1.51	2.23	1.69	......	4.8	4.8	4.9	4.9
7 days....	......	0.91	1.47	1.65	2.08	......	4.2	4.5	4.7	4.6
9 days....	......	1.03	1.73	1.98	2.39	......	4.1	4.7	5.0	4.6

* Aliquots of 15 seeds or seedlings.

The same deviation from the results of the previous experiment, probably due to the same cause, is shown by the potassium content (table IV) of the seedlings. Although the ungerminated seeds of the seven and nine day sets in the dense series showed greater potassium losses than in the previous experiment, the seedlings of the same sets and the same series not only made no gains in potassium, but even showed losses which were appreciable in the seven day set. Evidently the conditions of these experiments were more favorable for the leaching of potassium from the ungerminated seeds as well as from the normal mother seeds, than for its absorption by the seedlings. The results in changes in the phosphorus content are in full

accord with those obtained previously, showing losses from ungerminated seeds in the seven and nine day sets, and only a slightly changed phosphorus content in the corresponding seedlings.

Appreciable losses of phosphorus occurred in the ungerminated seeds of the dense series in the seven and nine day sets; in all other cases the changes in the phosphorus content are but slight (table IV). There is more variation in the potassium content. The potassium figures indicate a race between the leaching of this element from the seeds and its absorption by the seedlings. The losses of potassium from the ungerminated seeds are greater in the seven and nine day sets of the dense series than in the corresponding sets of the sparse series. This is probably true also of the mother seeds attached to the growing seedlings. After five days, when conditions favoring the leaching of potassium had not developed fully, the seedlings of the dense series showed a slight increase in potassium over the stock seeds; the corresponding seedlings of the other series showed a loss. This was probably due to the difference in stand of the plants, which was somewhat more vigorous in the dense than in the sparse series. As the losses through leaching from the mother seeds in the subsequent periods increased in the dense series, while remaining stationary in the sparse series, the seedlings of the sparse series began to gain in potassium over the corresponding seedlings in the dense series, and at the nine day period showed a pronounced increase over the stock seeds, while the seedlings of the dense series showed a potassium content below that of the stock seed. Evidently absorption of potassium by the seedlings of the dense series did not keep up the race with leaching of the same element from the mother seeds.

Discussion

The results of the two experiments show that wheat seedlings, within the age limits in which they are used for experimental purposes, while changing but slightly in phosphorus content during germination, vary appreciably in nitrogen and potassium content from the stock seed from which they were obtained, depending upon their age and the conditions under which they were grown. Seven days from the time the seeds were set to germinate, when the tops were about 3 inches high, both experiments showed an increase of about 10 per cent of nitrogen over the stock seeds when they were

germinated in a dense continuous mass of one layer. LIPMAN and TAYLOR[4] report direct fixation of atmospheric nitrogen by wheat plants, grown without added nitrogen, basing their conclusions on gains over the stock seed fluctuating between 20 and 50 per cent of the original nitrogen content. An increase in the content of the seedlings during germination of the same magnitude as obtained in these experiments would have cut down the reported gains. The main thesis of this paper would seem to be well founded, therefore, the results indicating that in plant nutrition studies the seedlings with which the experiments are studied are a safer standard of comparison than the stock seed from which they were grown.

The losses of nitrogen, potassium, and phosphorus observed at certain phases of the experiments were probably due largely to leaching, and partly perhaps to absorption by microorganisms. Potassium could not have been lost in any other way; no nitrogen (see footnote 1) in gaseous form is lost during germination and early life of wheat seedlings; and volatile phosphorus compounds are very limited in number, and probably would not develop under the conditions of these experiments.

The reported gains of potassium come principally from leaching from ungerminated seeds, and in part perhaps from the potassium content of the tap water in the first experiment. The reported gains in nitrogen also come mostly from ungerminated seed leachings. Possible direct fixation of nitrogen, as reported by LIPMAN and TAYLOR, could hardly account for such relatively large gains in a short time. Furthermore, the possibility of direct fixation could not very well be harmonized with the pronounced difference in nitrogen gains between the seedlings of the dense and those of the sparse series. There is no apparent reason for such a difference in fixing power between the two sets of seedlings, both of which were normal. The possibility of nitrogen fixation by microorganisms, however, is not excluded, as the wheat leachings formed a very good medium, especially in the dense series, for microorganic growth.

The results reported here have a bearing also on the possible deficiency of sterile seeds in plant food elements. The nitrogen, potassium, and phosphorus contents of the seeds which failed to

[4] LIPMAN, C. B., and TAYLOR, J. K., Do green plants have the power of fixing elementary nitrogen from the atmosphere? Jour. Franklin Inst. **198**:475–506. 1924.

germinate were generally lower than those of the stock seed, especially in the case of potassium. Is it possible that besides the losses by leaching which are inferred from corresponding gains, sterile seeds are naturally deficient in these elements? The results of these experiments seem to lend no support to the possibility. Losses of phosphorus from the ungerminated seeds were very slight in the earliest period but increased subsequently. The consistent differences in the potassium content of the ungerminated seeds between the dense and sparse series would also tend to show that the losses were largely due to leaching. The possibility that sterile seeds are deficient in some plant food elements is not excluded, but more experimentation would be necessary to prove or disprove this.

Summary

1. Wheat seedlings, between the age limits in which they are generally used for experimental purposes, differed in composition from the stock seed from which they were obtained. They either lost or gained potassium and nitrogen, depending upon their age and the conditions under which they were grown, but changed little in phosphorus content.

2. Seven days from the time the seeds were set to germinate, or three to four days after germination, the nitrogen content of the wheat seedlings was about 10 per cent greater than the nitrogen content of the stock seed, when it was spread to germinate in a dense continuous layer.

3. The results indicate that the gains in nitrogen and potassium were due chiefly to the absorption of materials leached from the ungerminated seeds.

4. The ungerminated seeds consistently lost more potassium, nitrogen, and phosphorus than did the seedlings. The results, however, do not settle the question as to whether sterile seeds may be naturally deficient in these elements.

5. It is recommended that in plant nutrition studies the seedlings with which the experiments are started, rather than the stock seed from which they were grown, be used as a standard of comparison.

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EVAPORATION IN THE SCIRPUS VALIDUS AND S. AMERICANUS ASSOCIATIONS[1]

FRANK C. GATES

(WITH PLATE VIII AND ONE FIGURE)

Introduction

In connection with some work endeavoring to discover the reason for the succession of *Scirpus validus* by *S. americanus*, the replacement of an association of relatively high plants by one of lower plants, atmometers were used in the vicinity at different levels in different years. The present paper is a report of the evaporation conditions found at the tension line between the two associations, and at the levels of the tops of the culms of the two species of *Scirpus*. Following some preliminary work, carefully planned series were used in the summers of 1921 and 1922. The writer is indebted to Miss ALICE E. KEENER for nearly all of the reading of the instruments during the first year; and, during the summer of 1922, when a more extensive series was used, to Mrs. RUTH H. WEST for the same assistance. The work was carried on at the University of Michigan Biological Station at Douglas Lake, Cheboygan County, Michigan.

Methods

Standardized Livingston spherical atmometers were used throughout the course of the work, one of each pair a white sphere and the other a blackened sphere. During 1921 the mercury glass wool valves of Livingston-Thone[2] were employed, while during 1922 the Gates' modification of the Musch rain correcting valve was utilized. In all cases the atmometer was set in an ordinary jar through rubber stoppers, in which there was also a tube projecting above the rubber stopper. The instruments were filled to the mark on this tube, which when in ordinary use was covered with a loose fitting cap. Burettes reading to 0.05 cc. were employed. In setting

[1] Contribution no. 245 from the Botanical Laboratory of the Kansas State Agricultural College.

[2] THONE FRANK, Rainproofing valves for atmometers: A résumé. Ecology 5:408–414. 1924.

up the instruments, stakes were driven into the ground, cross pieces arranged at proper heights, and the bottles wired on so that the atmometer cups were exposed at the desired elevation. Where two levels were maintained, a separate stake was utilized for the lower one, and was located a very short distance south of it, so that there was no possibility of shade from any part of the apparatus falling upon any of the cups. These were read at moderately regular intervals and the data recorded.

Description of stations

During 1921 two sets were used in Deer Bay, in the first of the crescent-shaped beach pools (Pl. VIII). These beach pools were separated from Douglas Lake by a narrow sand bar, which in years of high water might be covered. The vegetation of most of the beach pools was a very dense growth of *Scirpus americanus*, in a few parts of which, especially toward the western corner, moderate sized patches of *S. validus* were present. At the edges of the pools, and up to the ridges separating them there might be examples of *Salix-Cornus* thickets, and scattered throughout, where it was possible for them to grow, were a few secondary species, all much shorter than either *Scirpus*, and consequently of no effect upon evaporation from the atmometers.

One set was used in the western part of the largest of these beach pools and a second in the central part. In the first case the set was located at the tension line between rather large patches of *S. validus* and *S. americanus*, while the other was located at the edge of a moderate sized patch of *S. validus* in an extensive area of *S. americanus*.

During 1922 several sets were used, one of which was in Deer Bay, located on the same stakes as were employed in 1921. Another set was used in a cove west of Grapevine Point, where *S. americanus* forms rather extensive growths running from the very shallow water back to the land, and in some cases quite back to the fringing ridge, which is either shrub or tree covered. *S. validus* is now rather scarce here, having suffered severely a few years ago from ice work. The atmometers were set up in the same manner, on the tension line between small patches of *S. validus* and the edge of a rather large

patch of *S. americanus*. At the time the apparatus was set up in June, the water of the lake was about 15 cm. deep, but the lake level lowered during the course of the summer, so that the stakes were on land at its close. In this location there was marked protection from southerly winds, as the bluffs behind rose to heights of 6–8 m. Further, there was a moderate amount of protection from northerly winds, on account of the shelving beach which projects out some hundred or more meters and breaks the effect of storm waves.

At the west end of the lake two sets were used, one in the cove west of Maple Point, and the other in the open lake to the east of Maple Point. In the case of the cove, the atmometers were placed at the edge of a sparse growth of *S. validus* where it approached the *Eleocharis* consocies of the *S. americanus* association. In this situation there was a certain amount of protection from most of the northerly winds, and the location of the cove is such that wave action was very slight.

The set placed east of Maple Point was located at the base of Phragmites Flat, in a medium dense growth of *S. americanus* at the edge of a moderate sized patch of *S. validus*. In this situation protection was afforded from west winds and less so from north winds, but from all other directions there was nearly a full sweep of the lake.

During 1920 and 1921, atmometers were placed in *S. validus* on the Big Shoal, which projects somewhat more than a kilometer out into the lake. The station was fully exposed to winds from all directions, and was well calculated to show the extreme of evaporation conditions which *S. validus* withstands. Here *S. validus* was present without associated species of higher plants. It was growing in water 1.4–1.8 m. in depth, and projected above this about 1.2–1.5 m. Water of such depth makes it entirely impossible for *S. americanus* to develop.

During July and most of August, 1920, the evaporation from a white atmometer here averaged 34.54 cc. per day. In the season of 1921, a white atmometer averaged 42.88 cc. per day from July 2 through August 16. Up to August 8 a black atmometer averaged 55.73 cc. per day, while a white one averaged 45.4 cc. per day for the same period. These data indicate that *S. validus* can withstand high evaporating conditions of the air.

The standard atmometer is that located at the Biological Station to permit comparison between different seasons.

Results and interpretations

Comparing the actual rates of evaporation, it is obvious that evaporation varies from year to year and from station to station over a rather wide range. As the averages are moderate to high, it is evident that *Scirpus* is well able to withstand high evaporating conditions of the air. Its roots are submerged, so that apparently there is a superabundance of water to supply transpiration. As was to be expected, the black atmometers uniformly showed a higher rate of evaporation than the white at the same levels. This was most pronounced of course in dry sunny weather, usually between half again and as much again as evaporated by the white atmometers.

Variation between the seasons was accompanied by similar variation in rates; for instance, the white standard rates in 1921 and 1922 were 29.44 and 22.59 cc. per day respectively, while the atmometers in Deer Bay which were run in both these years gave 19.70 and 15.28 cc. per day for the same times. In a season of less evaporation, the ratio of the black to the white atmometer was less (1.69 in 1921 against 1.25 in the cooler 1922).

The atmometer at the higher level (that of *S. validus*) always evaporated more than the corresponding one of the pair at the lower level (that of *S. americanus*). The difference of 0.6 m. from about 0.4 to about 1.0 m. made a difference of from 1.16 to 1.37, compared with unity at the lower level, for different situations in the cases of the white atmometers, and from 1.07 to 1.40 similarly with the black atmometers. A black atmometer at the lower level usually showed a higher rate of evaporation than a white atmometer at a higher level, indicating that within the difference of elevation concerned (about 0.6 m.) the insolation was of greater importance than any other factor.

Comparing the black to white ratios at different levels, it was noted that the ratio, with a single exception, was higher at the lower level. This was probably because the extra energy of insolation could make itself felt strongly, while, in the presence of a decreased circu-

lation at the lower level, the rate from the white atmometer was diminished. The greatest difference was at Deer Bay in 1922, when the black to white ratio at the higher level was 1.25, while at the lower level it was 1.57.

At the level of the culms of *S. validus*, there was no significant change of ratio with the standard during the course of the season. The variations above and below the averages are easily accounted for by the variabilities of the weather conditions, especially the amount and direction of the wind and the moisture in the air.

Comparisons of the results obtained at the level of the culms of *S. americanus*, however, showed a tendency to be lower later in the season. This would be expected, because of the increase in density of the vegetation during the season; but the fact that it was so small, and in the more exposed habitats so uncertain, simply means that the increase in height and number of the slender flexible upright *Scirpus* culms is but slightly effective in decreasing the air currents and shade, thus reducing the evaporation below that "normal" for a station at the 0.4 m. level. Further, the normal habitat of both species of *Scirpus* is quite an exposed one, especially so in the case of *S. validus*.

While the ratios of either the white top or the white bottom to the standard were usually rather closely similar, although of different amounts, the introduction of rainy days, or especially a rainy period, would throw these ratios greatly to one side in the cases of the black atmometers as compared with the white ones.

In the stations employed during the course of the atmometer work, it did not appear that the plants of either species of *Scirpus* were growing under air conditions that were in the slightest affecting them because of injurious lack of water. The action of other factors was often quite obvious. With respect to diminished evaporation, both *S. americanus* and *S. validus* grew in competition with *Salix-Cornus* thickets, if these thickets approached over wet ground. Actual shade eliminated *S. americanus* quickly, but *S. validus* persisted in several cases into thickets of moderate density, and even into a light forest in rare cases. In such places the evaporation is always conspicuously lower than in the open. In all cases, however, the roots of these species of *Scirpus* were at or below the water table

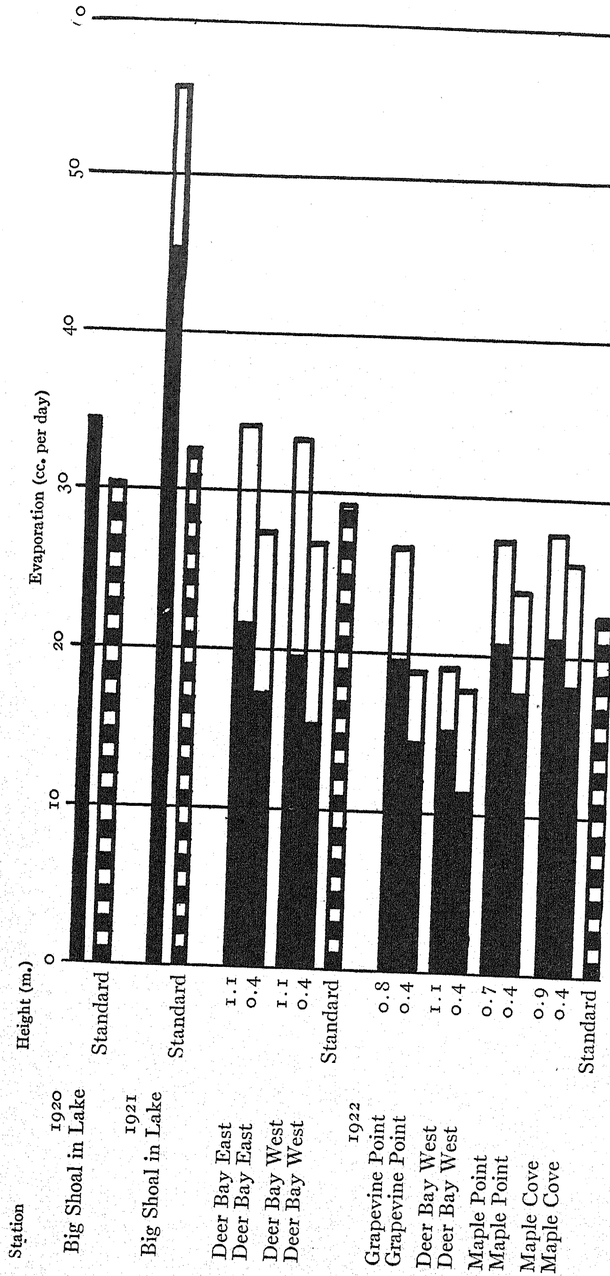

FIG. 1.—Diagram of average daily evaporation from atmometers in *Scirpus* association (upper at level of *S. validus*, lower at level of *S. americanus*), and from the standard at Biological Station for same periods; solid portion of line indicates white atmometer, black edged projection indicates additional evaporation from black atmometers of the pairs, and broken line indicates standard (white) for of time associated group.

TABLE I

AVERAGE AND EXTREME VALUES OF EVAPORATION AROUND S. VALIDUS AND S. AMERICANUS AND RATE OF STANDARD AT BIOLOGICAL STATION DURING SAME TIME

Year	Station	Days of record	Top*				Bottom*				Standard during same period
			Black		White		Black		White		
			Average†	Extreme†	Average	Extreme	Average	Extreme	Average	Extreme	
1920	Scirpus in lake	46	......	....	......	....	......	....	34.54	$66.89^{1.0}$	30.44
1921	Scirpus in lake	38	......	....	......	....	55.73	$72.72^{2.5}$	45.40	$55.58^{3.5}$	32.68
1921	Deer Bay east	52	34.24	$52.93^{2.5}$	21.62	$33.62^{2.5}$	27.63	$44.96^{2.5}$	17.23	$26.69^{2.5}$	29.44
1921	Deer Bay west	52	33.30	$57.08^{3.5}$	19.70	$34.69^{1.9}$	26.88	$46.33^{1.9}$	15.54	$27.11^{1.9}$	29.44
1922	Grapevine Point	50	26.76	$48.02^{2.9}$	19.72	$36.20^{2.9}$	19.11	$29.16^{4.5}$	14.39	$23.94^{2.9}$	22.59
1922	Deer Bay west	50	19.14	$33.14^{2.9}$	15.28	$28.21^{2.9}$	17.95	$30.61^{2.9}$	11.40	$23.82^{2.9}$	22.59
1922	Maple Point	49	27.39	52.80^{2}	20.82	43.83^{2}	24.14	44.50^{2}	17.84	36.49^{2}	22.59
1922	Maple Cove	49	27.75	78.10^{2}	21.19	63.49^{2}	25.93	72.80^{2}	18.20	58.20^{2}	22.59

* Top designates level of *S. validus* culms (varying 0.7–1.1 m.); bottom designates level of *S. americanus* culms (varying 0.35–0.45 m. above water).

† The average during the summer is expressed in cc. per day, with the Extreme columns expressing the extreme rate during a period, the duration of which in days is expressed by the exponential figure to the right.

level. So long as this was true, it appeared to make no difference whatever with respect to the evaporating condition of the air in itself.

Summary

The evaporation results herein reported were obtained in connection with some studies on the *S. validus* and *S. americanus* associations, in the vicinity of the University of Michigan Biological Station on Douglas Lake, Cheboygan County, Michigan, during 1920, 1921, and 1922.

From the fact that *S. validus*, provided its roots are at or below the water table level, will grow under a wide range of evaporating conditions, including exposure to those both less and more severe than under normal successional conditions, it is evident that evaporation has nothing to do with the succession from the *S. validus* to the *S. americanus* association.

The actual averages and upper extremes at the height of the *S. americanus* culms and at the height of those of *S. validus* are shown in table I and fig. 1. Within the limitations of this experiment at least, the blackened condition of the atmometer was more potent in increasing the rate of evaporation than elevating the instrument.

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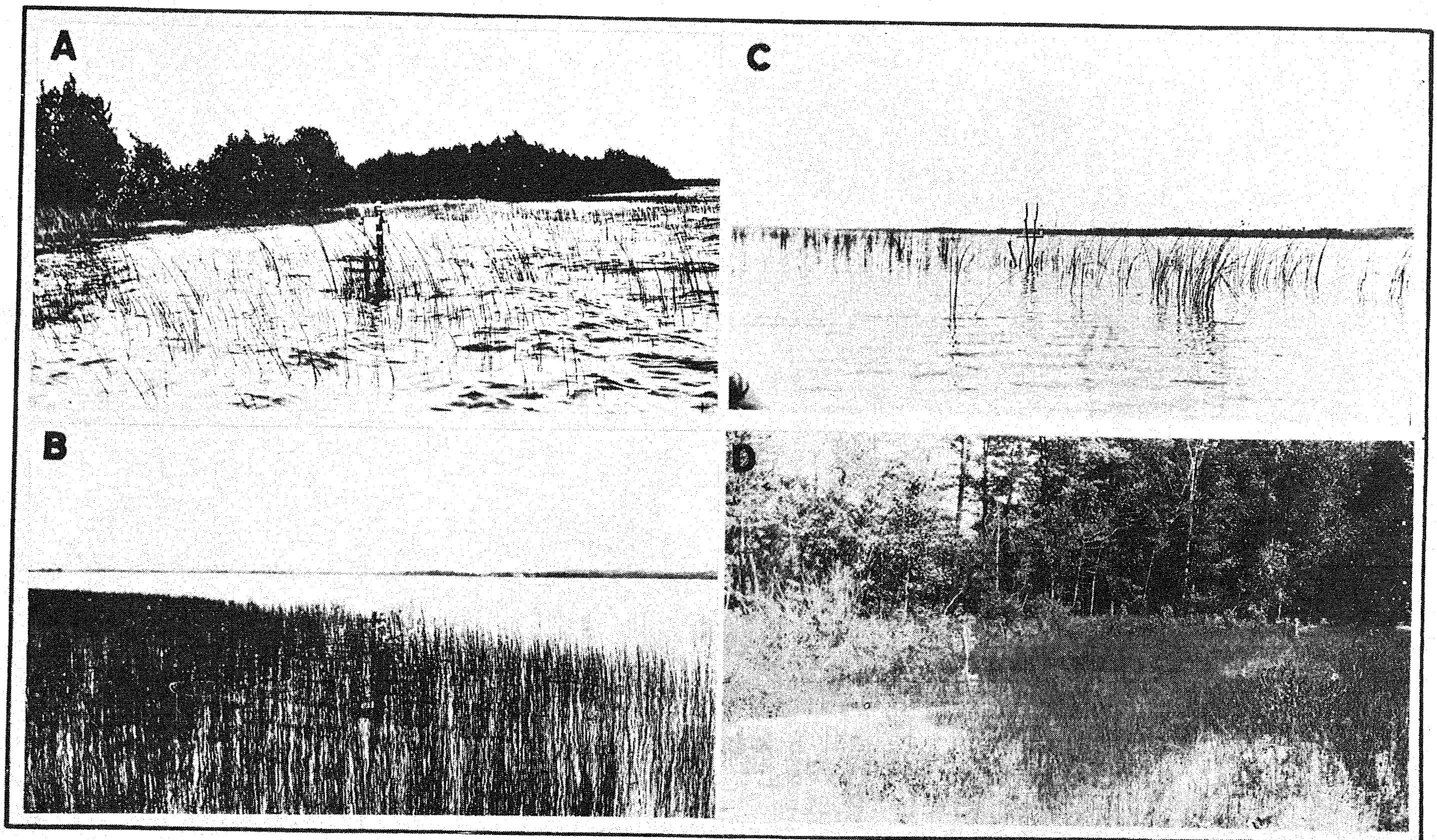
EXPLANATION OF PLATE VIII

PLATE VIII

Atmometer set-ups in *Scirpus* vegetation in Douglas Lake, Michigan, except for *C*, on tension lines between *S. validus* and *S. americanus* associations: *A*, view to east of Maple Point, showing *Scirpus* zone near beginning of growing season (June 25, 1922); *B*, same as *A*, but taken from shore after season's growth had been completed (August 11, 1922); *C*, view on Big Shoal, showing open development of *S. validus* association in water over 1.6 m. deep (August 3, 1921); *D*, view in Deer Bay, showing dense development of *Scirpus* associations (July 1, 1921).



GATES on SCIRPUS

INHERITANCE OF PUBESCENCE IN PHARBITIS NIL

Yoshitaka Imai

The stem of the common strains of *Pharbitis Nil* (the Japanese morning glory) is more or less silver gray in color in reflected light, on account of the somewhat dense hairs growing downward on its surface. In one of my pedigrees, no. 65, the development of the hairs on the stem is very poor, however, and the stem which grows out in the autumn is almost free of hairs. Because of this condition the stem and the foliage of the strain are of vivid color. Roughly speaking, the strain may be said to have a smooth stem.

Crossing this strain with the hairy normals, I obtained F_1 plants having a hairy stem, and they gave rise to the F_2 offspring composed of the hairy and the smooth stems in nearly the usual ratio. With these results, we may assume that the smooth stems which are produced breed true to type in the subsequent generation. Some of the F_3 families of these smooth stems, however, gave unexpected results, segregating into smooth and hairy stems. Thus the hairy condition behaves either as a dominant or a recessive to the smoothness in the descendants of the same cross.

The smooth condition is commonly found to be recessive to the hairy state in many different species of plants. According to Miss Saunders (2), and Miyake and Imai (1), however, the hair of the stem in the foxglove is transmitted as a recessive allelomorph to the so-called smoothness. The smooth stem in this plant is not entirely or nearly free from hairs, only the upper flowering part of the stem being almost smooth. In *Pharbites Nil* the relative development of hairs on the stem is represented in a manner somewhat similar to that of *Digitalis*, but the genetic behavior is more complicated, on account of the occurrence of a dominant hairy stem.

Experimental results

The F_1 plants obtained by crossing a smooth stemmed strain (65) and two hairy stemmed specimens (326 and 220) had quite

hairy stems; their reciprocal matings gave no different results. Table I gives the results obtained in raising the F_2 offspring.

TABLE I

Cross	Hairy stem	Smooth stem	Total
65×326.......	93	30	123
65×220.......	141	25	166
Total.........	234	55	289
Expected (13.3)	234.81	54.19	289

TABLE II

F_3 DATA OF CROSS 65×326

Character of F_2	Pedigree number	Hairy stem	Smooth stem	Total
Hairy stem	Total of 38 pedigrees	1153		1153
	33	19	6	25
	36	19	7	26
	41	?	?	(101)
	42	11	3	14
	43	12	3	15
	46	11	7	18
	47	23	8	31
	49	38	6	44
	54	7	4	11
	57	42	14	56
	61	18	9	27
	62	29	14	43
	66	43	11	54
	67	18	6	24
	69	4	1	5
	71	6	4	10
	Total	300	103	403
Smooth stem	Total of 8 pedigrees		236	236
	28	13	29	42
	29	5	10	15
	30	2	8	10
	31	22	62	84
	34	7	19	26
	37	1	6	7
	39	1	3	4
	40	4	12	16
	63	4	11	15
	70	5	14	19
	73	4	8	12
	Total	68	182	250
	Expected	62.5	187.5	250

The segregating number is not very far from the simplest ratio of 3:1, and we might think that the case is one of monohybrid inheritance, but the case is not so simple when the F_3 data are examined. The actual segregation may be a modified dihybrid ratio of 13:3, the former having hairy stems and the latter smooth ones. The F_3 results obtained from a cross, 65×326, are shown in table II.

Offspring of hairy stemmed F_2.—Roughly speaking, the results of the hairy stemmed F_2 are divided into two classes, the one breeding true and the other segregating into hairy and smooth stems. The segregating number in total nearly corresponds with that calculated on the basis of 3:1. Theoretically, however, the average proportion must be $3>1$, as will be shown in the next section.

Offspring of smooth stemmed F_2.—The F_3 families of the smooth stemmed F_2 are equally divided into two types, those breeding pure and those producing hairy stems. Now we have a case of reversal segregation, namely, the segregating smooth stems produce the hairy stems. The segregating hairy stems are in the proportion of 27 per cent of the total number, consequently the case may be considered as practically 3:1 segregation.

Discussion

In summing up, the results obtained by the cross 65×326 may be shown graphically as follows:

P	F_1	F_2		F_3
Hairy stem		76% Hairy stem	70%	100% Hairy stem
			30%	74% Hairy stem 26% Smooth stem
×	100% Hairy stem			
Smooth stem		24% Smooth stem	42%	100% Smooth stem
			58%	73% Smooth stem 27% Hairy stem

The dominant nature of the hairy condition can be learned by the fact that the F_1 plants all had hairy stems, and they produced smooth stems in about one-fourth of the total F_2. Some smooth stemmed F_2 plants, however, produced offspring consisting of the smooth and the hairy stems in the proportion of 3:1. The result shows entirely reverse behavior in the segregation of the similar characters.

Now let us assume two allelomorphic pairs of factors in relation to the development of the hair on the stem: (1) H_s, h_s.–H_s inhibits

the production of hairs on the stem, while its recessive factor h_s is responsible for the hairy stem; (2) H_h, h_h.—H_h acts as an inhibitor to H_s, making the stem hairy.

Then the genetic composition of the parental hairy stems should be $h_sh_sH_hH_h$, while the smooth partner is considered to be $H_sH_sh_hh_h$. In the consideration of the former composition two alternatives may be offered, $H_sH_sH_hH_h$ or $h_sh_sh_hh_h$, but neither of them can be thought of as the actual case, since the F_1 plants must have a double heterozygotic constitution. The free combinations of the factors in the F_2 generation should give:

Reference	Genetic composition	Its ratio	Character	Its ratio
A	$H_sH_sH_hH_h$	1	Hairy stem	13
	$H_sh_sH_hH_h$	2		
	$h_sh_sH_hH_h$	1		
	$h_sh_sH_hh_h$	2		
	$h_sh_sh_hh_h$	1		
B	$H_sH_sH_hh_h$	2		
	$H_sh_sH_hh_h$	4		
C	$H_sH_sh_hh_h$	1	Smooth stem	3
D	$H_sh_sh_hh_h$	2		

The ratio of the hairy and the smooth stems is 13:3. With such an expected ratio as a basis, the observed and the theoretical numbers approximately corresponded, as was shown in the bottom line of the F_2 table. Out of seven hairy F_2 genotypes, five grouped in *A* should phenotypically breed true to the hairy stem, whatever their constitution may be. The remaining two types grouped in *B*, however, should produce segregating families; the one, single heterozygotic type, will produce hairy and smooth stems in the ratio of 3:1; while the other, double heterozygotic type, may give the two forms in the proportion of 13:3. The two types of ratio, 3:1 and 13:3, are not so different that they can always be distinguished easily one from the other with the numbers which were observed. Those families which produced some smooth stems are then considered to be composed of two types of segregation; so the ratio in the total number should be $3>1$, but not 3:1. The actual ratio, however, was nearly 3:1, not $3>1$. This unexpected result may be partly accounted for at least by the difficulty of distinguishing between the two forms. Although in the majority of the cases the

classification was not hard, it was sometimes difficult. The extreme case is exhibited in family 41, of which I failed to take reliable records as to the segregating characters. Such difficult conditions for identification seem to admit of some qualifications in judging the data. Out of two smooth stemmed F_2 genotypes one (*C*) should phenotypically breed true to the smooth stem, while the other (*D*) will produce smooth and hairy stems in the ratio of 3:1. These expectations coincided nearly with the actual data.

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BRIEFER ARTICLES

GERMINATION OF SEEDS OF PLANTS NATIVE TO DUTCHESS COUNTY, NEW YORK[1]

Appreciation of the value of the propagation of native plants is growing rapidly among those interested in landscape gardening and in plant conservation. The attractiveness of some of these plants makes an increased supply of them desirable, while some of the more striking will soon become extinct if no effort is made to check the destruction of the natural supply.

The department of botany at Vassar College, in cooperation with the Conservation Committee of the Garden Club of America, is working upon this problem, from the standpoint of obtaining information in regard to the germination of seeds of native plants. The growth of native plants from seed insures the widest utilization of such plants and the least loss. Seeds of some introduced plants have been included in this study.

Treatment of seeds

The collections were made in September, October, and November of the years 1922, 1923, and 1924. Dry fruits were shaken or pounded to loosen the seeds, which were then sifted out, and stored in paper sacks in a dry place at room temperature (20°–25° C.) until needed. Pulpy fruits were dried and stored in the same way. When the seeds were to be used, the fruits were soaked in water, mashed, and the pulp washed away. Sterile water only was used to rinse the seeds. In the experiment where nipping was used, the seed coats were broken through to the endosperm by means of scissors before the seeds were sterilized. Petri dishes were fitted with pieces of filter paper and cotton, and then sterilized in an oven for two hours at 160° C. The sterilized seeds were spread on the filter paper which had been moistened with sterile water.

In the experiments made from October, 1923 to May, 1924, twenty-five seeds were used in each dish. In tests made from October, 1924 to March, 1925, one hundred seeds were used in each dish. Two dishes were used in each experiment, both being kept at 20°–25° C., but one in diffuse

[1] This investigation was conducted under the direction of Dr. Edith A. Roberts, whose aid and encouragement are gratefully recognized by the writer.

light and the other in the dark. The paper was moistened with sterile water every two or four days as needed.

The experiments set up from October, 1923 to March, 1924 were observed from the date of setting up until June 1, 1924. Those set up during April and May, 1924, were observed until June 10, 1924. Those set up from October, 1924 to March, 1925 were observed until the latter part of April, 1925. Records of germination were made from two to eight days apart.[2]

Results

TABLE I

SEEDS UPON WHOSE GERMINATION LIGHT HAS A BENEFICIAL EFFECT

Name	Year collected	Light		Dark	
		Days	Percentage germination	Days	Percentage germination
Anaphalis margaritacea	1922	4–9	18	5	12
	1924	4–12	50	4–12	12
Apocynum cannabinum	1923	6	44	6	4
	1924	5	13	6	12
Anemone virginiana	1924	29–75	66	181	0
Cuscuta arvensis	1923	5–133	39	4–90	18
Daucus Carota	1922	17	44	27	0
	1924	91	1	151	0
Dianthus Armeria	1922	9–16	88	4	8
	1923	4–33	79	4–82	31
Echium vulgare	1924	2–7	91	158	0
Eupatorium purpureum	1922	12	20	26	0
Gentiana Andrewsii	1923	95–209	0	95–219	0
	1924	14–30	80	20–50	18
Helenium autumnale	1923	12–61	19	21–243	0
Hesperis matronalis	1923	3–51	80	5–12	28
Heuchera americana	1923	15–38	47	14–47	12
Linaria vulgaris	1922	11–17	24	31	0
	1923	9–59	15	20	1
	1924	13–26	21	181	0
Lobelia cardinalis	1922	9–12	95	9–22	0
Lythrum Salicaria	1922	6–10	84	6	4
	1923	7–82	67	6–10	9
Mimulus ringens	1923	14–28	56	28	0
Pentstemon Digitalis	1923	14	32	28	0
Penthorum sedoides	1922	18	52	28	0
	1923	18–181	23	28–233	0

[2] Standards in judging the effect of light are as follows: (1) If there is more germination in light than in darkness, light is said to be beneficial to germination; (2) if the germination in darkness is more than 15 per cent greater than that in light, darkness is said to be beneficial to germination; (3) if the average germination in light is within 5 per cent of being equal to that in darkness, and the germination in no individual experiment differs by more than 20 per cent between light and darkness, light is said to have no marked effect upon germination.

TABLE I—*Continued*

Name	Year Collected	Light		Dark	
		Days	Percentage germination	Days	Percentage germination
Physalis virginiana	1923..........	3–86	59	10–119	43
Phytolacca decandra	1923..........	36	72	84	0
Plantago major	1923..........	10	16	19	0
Potentilla recta	1923..........	7–165	5	7–68	12
Potentilla arguta	1924..........	3–12	52	8–88	9
Pseudera quinquefolia	1923..........	13–32	48	12–34	7
Radicula palustris	1923..........	8–76	30	30–233	0
Rhamnus cathartica	1924..........	23–59	44	23–59	24
Saponaria officinalis	1924..........	6–46	30	6–24	4
Scrophularia leporella	1923..........	19–28	44	28	0
Scutellaria lateriflora	1922..........	11	88	21	0
	1923..........	11–94	21	20–227	0
Solidago nemoralis	1923..........	....	64	109	0
Solidago rugosa	1923..........	91	52	109	0
Spiraea tomentosa	1924..........	15–41	50	41	2
Tanacetum vulgare	1923..........	3–23	96	5–8	68
Verbascum Blattaria	1923..........	2–73	59	4–56	34
	1924..........	24	70	24	4
Verbena hastata	1923..........	28	33	0	0
Verbena urticaefolia	1923..........	16–97	17	91–225	0

TABLE II

SEEDS UPON WHOSE GERMINATION DARK HAS A BENEFICIAL EFFECT

Name	Year collected	Light		Dark	
		Days	Percentage germination	Days	Percentage germination
Polygonatum biflorum	1923..........	9–146	13	9–146	32
Smilacina racemosa	1923..........	88–240	0	72–147	16
	1924..........	199	0	38–63	5
Smilacina stellata	1923..........	37	4	6–37	84

TABLE III

SEEDS UPON WHOSE GERMINATION LIGHT HAS NO MARKED EFFECT

Name	Year collected	Light		Dark	
		Days	Percentage germination	Days	Percentage germination
Achillea millefolium	1922	21	64	12	96
Asclepias tuberosa	1923	8–33	23	12–188	24
	1923	4–33	77	3–36	73
	1924	6–20	60	6–39	90
Arisaema triphyllum	1923	57	96	63	96
Cassia marilandica	1923	34	20	34	24
Cichorium Intybus	1923	54	84	29	72
Epilobium molle*	1923	68	88	163	80
Gnaphalium polycephalum	1922	5–24	52	5–36	35
Lilium philadelphicum	1923	109	84	91	84
Oenothera biennis	1923	11	72	15	60
Physalis virginiana	1923	16	72	56	56
Rudbeckia hirta	1923	5	56	12	52
Silene latifolia	1923	9	92	13	88
Solanum Dulcamara	1923	5	92	13	96
Tanacetum vulgare	1923	7	92	8	88
Tragopogon pratensis	1923	10	80	10	100
Veronica virginica	1923	28	44	68	32
Viburnum acerifolium	1923	236	64	200	64
Viburnum prunifolium	1923	177	55	178	52

* In this instance the dark plate was brought into light at the 152d day.

TABLE IV

SEEDS UPON WHOSE GERMINATION NIPPING HAS A BENEFICIAL EFFECT

Name	Not nipped				Nipped			
	Light		Dark		Light		Dark	
	Days	Percentage germination	Days	Percentage germination	Days	Percentage germination	Days	Percentage germination
Apocynum cannabinum	6	44	6	4	6	72	6	40
Cassia marilandica	6	4	12	4	7	84	3	68
Ceanothus americana	5	4	8	8	26	0	8	36
Dioscorea villosa	30	0	30	0	30	4	30	16
Phytolacca decandra	30	0	30	0	3–18	40	7–12	48
Rhus copallina	28	0	28	0	10	24	19	48
Rhus glabra	28	0	28	0	18	48	14	28
Sagittaria latifolia	......	......	......	......	64	77	104	93
Solidago bicolor	26	8	17	16	26	28	12	8
Solidago nemoralis	26	0	12	0	26	20	12	8
Verbena hastata	15	18	33	0	5–12	32	5–7	40
Verbena urticaefolia	91	0	91	0	5	24	5	56

The highest percentage of germination (that is, 20 per cent in 33–67 days) of seeds of *Mitchella repens* occurred when the seeds were treated as follows. The berries were kept in the dark at a temperature of 5°–10° C. for six weeks. The berries were then mashed and the pulp floated from the seeds. Then the seeds were nipped and placed under germinative conditions in the dark.

The best percentage of germination (that is, 52 per cent in a range of 15–49 days) of seeds of *Celastrus scandens* occurred when the seeds were treated as follows. The seeds were collected from the vines on January 31. They were removed from their coats on March 9 and placed under germinative conditions in the dark at a temperature of 5°–10° C.—ESTHER MITCHELL, *Department of Botany, Vassar College, Poughkeepsie, New York.*

CURRENT LITERATURE

BOOK REVIEWS

Chemical basis of growth and senescence

An extensive literature concerning the growth rate of animals and plants has sprung into existence since ROBERTSON (1908) first suggested that growth curves of organisms are similar to the rate curves of autocatalyzed monomolecular chemical reactions. This literature has been summarized by ROBERTSON[1] in book form, and his theory of autocatalysis of growth is presented fully, with such evidence as seems to support his interpretation. The introduction presents growth as a self-accelerated process, carried on by some "master reaction," which is conceived as probably multimolecular, although protoplasmic breakdown may be monomolecular. The first part of the growth curve, when growth is increasing in rate, is called the autokinetic phase, and the later part, where the growth rate is decreasing, is called the autostatic phase of growth.

The succeeding chapters consider the growth cycles in man, animals, and plants, and in unicellular organisms. The chapter on growth of unicellular forms considers allelocatalysis, and the significance of the lag period. The substrates of growth, inorganic salts, amino acids, and accessory vitamines as distinct from the catalysts of growth are considered in two chapters.

The autostatic phase is treated in several chapters dealing with such retarding influences as bodily dimensions, physiological differentiation, senescence, the influence of differentiation on the distribution of nutrients, metabolic rates, etc., and the relation of nutrient levels to growth rates.

The concluding chapters deal with hyperdifferentiation, the action of special chemical agents, such as lecithin, thyroxin, and others, and growth and evolution. An appendix provides tables for the computation of curves of autocatalysis. The literature citations number well over 500.

Briefly stated, ROBERTSON conceives growth as an autocatalyzed process, the autocatalyst having its origin in the nucleus of the cell. This nuclear catalyst is partitioned between nucleus and cytoplasm and pericellular medium during cell division when the nuclear membrane breaks down. The autocatalyst may be the substance called "bios" by WILDIERS 25 years ago. As the autocatalyst accumulates with successive cell divisions, it comes finally to exist in excess in older cells. This accumulation of catalyst tends to suppress the formation of more catalyst, with accompanying retardation of growth rates, so that "the

[1] ROBERTSON, T. B., The chemical basis of growth and senescence. 8vo. pp. viii+389. *figs.* 45. Philadelphia: Lippincott.

rate of development of the community (of cells), therefore, reflects the rate of accumulation of the autocatalyst in the pericellular media."

That ROBERTSON thinks his theory may ultimately explain the riddle of development is indicated in his preface, in which he refers to BERGSON, who has expressed the belief that the scientific method can never provide a solution of this puzzling problem. He calls the book a preliminary essay toward that interpretation of development which BERGSON declares to be intellectually impossible.

Among plant physiologists there has also been much discussion, and the subject has been summarized by RIPPEL[2] for the higher and lower plants. He divides the discussion into two main sections following an introductory statement of viewpoints, the growth curve, and the curve of yield.

While it is of interest to express growth rate curves in terms of mathematical formulas, these attempts to express growth in terms of autocatalytic phenomena are leading us off into the wilderness. In the sense that an organism makes its own enzymes, growth may be autocatalyzed; but the S-shaped curve of growth rates, observed in nearly all studies, is in itself not evidence of autocatalyzed growth in the ROBERTSON sense at all.

Evidence is accumulating to show that the S-shaped curve of growth rates in plants can be explained on the basis of nutrition and correlation, with the decided advantage that there is tangible evidence to support the interpretations. The work of MURNEEK,[3] [4] and POPP (unpublished) indicates that the slow growth of early life is related to the cotyledonary food supplies. Growth speeds up as the leaves become able to manufacture increasing food supplies, but begins to decrease as soon as reproductive organs divert the nutrient supplies to the reproductive structures. We do not need to call upon hypothetical catalysts to account for such S-shaped curves of growth. We need decidedly less theorizing and more solid experimental evidence and hard-headed reasoning in connection with these growth problems.—C. A. SHULL.

Physical chemistry in biology and medicine

Physical chemistry has become an indispensable tool to the investigator of physiological processes. Any assistance to the physiologists who are attempting to make good the deficiencies in earlier training along these lines should be welcome. McCLENDON and MEDES[5] have prepared a book to meet the needs

[2] RIPPEL, AUGUST, Wachstumsgesetze bei höheren und niederen Pflanzen. Naturwissenschaft und Landwirtschaft. Heft 3. pp. 90. Freising-München. Datterer. 1925.

[3] MURNEEK, A. E., Correlation and cyclic growth in plants. BOT. GAZ. 79:329–333. 1925.

[4] ———, The effects of correlation between vegetative and reproductive functions in the tomato. Plant Physiology 1:3–57. 1926.

[5] McCLENDON, J. F., and MEDES, GRACE, Physical chemistry in biology and medicine. 8vo. pp. 425. Philadelphia: Saunders. 1925.

of those who are trying to master the fundamental applications of physical chemistry to biological investigations. The treatment of the subject is divided into two sections, physico-chemical and physiological.

The first group of chapters takes up the fundamental notions of mass, volume, atomic structure, colloidal state, intermolecular attractions, osmotic phenomena, dissociation, conductivity, mass action and equilibria, hydrolysis, hydrogen ion, buffers, indicators, and oxidation-reduction potentials. The treatment of these topics in some cases strikes the reviewer as too elementary to be very helpful, except in the very beginning of such study. This may not be such a serious fault, considering the group for whom it is intended; but the treatment does not go far enough into the theoretical considerations in many places. Numerous references, however, open the way to more serious usefulness of the methods of physical chemistry in biology.

The physiological section deals with the nature of radiant energy and photodynamic effects; atomic structure and physiological action; thermochemistry and calorimetry; colloidal state of biological materials; influence of hydrogen ion concentration on enzymes, bacteria, and in soils, water etc.; ionic equilibria in blood; osmosis; permeability; and surface forces. There are hundreds of references to the literature, mostly recent publications, and these help to make up for the inadequacy of treatment at various places. The aim of the authors is laudable, for it is much better that the inadequately trained investigator should be stimulated to make good his deficiencies, rather than to curtail and limit the field of research. The book should prove helpful to those struggling against limitations set by lack of training in the physico-chemical sciences.—C. A. Shull.

The cell wall

Perhaps the feature which most sharply distinguishes plants from animals is the presence as a rule of a cell wall in the former. Van Wisselingh,[6] whose researches on cork are well known, has recently gathered together what is known about this distinctive plant feature into a most convenient and useful volume.

The work is divided into seven chapters, dealing respectively with Cellulose, Hemicelluloses and pectic substances, Lignin and lignification, Suberin and cutin, Chitin, Mineral components, and Structure and growth. Each chapter is subdivided into a number of sections, each complete in itself and dealing with such topics as chemical composition, effect of reagents, decomposition products, and microchemical detection and distribution in nature. A most valuable feature is the extensive bibliography appended to each section. The literature is very well summarized down to 1921, and an *addenda* chapter brings it down into 1924, so that it is really quite up-to-date.

In such a work one might expect to find much space devoted to a discussion of the various kinds of cell walls and cell forms, but the author in his foreword

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[6] Van Wisselingh, C., Die Zellmembran. 8vo. pp. vii+266. Berlin: Gebrüder Bornträger. 1925.

definitely delimits his subject so as to exclude this descriptive detail, placing the emphasis rather on the physiological than the morphological side. The only notable omission which the reviewer has detected is the absence of any suggestion as to the origin of the constituents of the cell wall. It may be that the author felt it to be outside his province to inquire into the past history of the building stones found in the wall.

The author has not done further research on the adipo-celluloses since the end of the last century, but has been studying the growth and structure of cell walls more recently, so that the only new studies of his own are to be found in the last chapter. He has fairly and ably summarized the researches and theories of other workers in every phase of the subject, however, and has produced a very valuable source book for reference. An author-index as well as a subject-index adds to its usefulness.—H. S. WOLFE.

Fruit growing

Another excellent book has been added to the literature of horticultural science. Out of a rich experience CHANDLER[7] has prepared a work which should be read by all who are interested in the physiological phases of the life of fruit trees, and particularly by those who expect to devote themselves to fundamental research underlying the practical arts of orchard management and fruit production.

The early chapters deal with the growth habits of fruit trees, the factors influencing bud formation, and the problem of the rest period or dormancy. The student will find in these chapters well balanced and discriminating discussions of the conditions which influence fruit bud formation, such as the carbohydrate-nitrogen relationship, ringing, root pruning, etc. Succeeding chapters deal with root and soil problems; water relations, including the responses of fruit trees to irrigation practices; and the management of orchard soils. Fruit setting, self sterility, fruit thinning, and especially pruning of trees receive careful and detailed consideration. The later chapters are devoted to the climatic responses of fruit trees, particularly light and heat responses, and the physiological changes associated with ripening, harvesting, and storage of fruits.

Most of the 27 chapters close with a summary, which aids materially in maintaining a well balanced point of view on the part of students. There is an extensive bibliography of nearly 1400 titles at the close of the work. The citations are arranged by subject to correspond to the chapters, and offer a well chosen introduction to the literature of this field.

The book is very meritorious from the standpoint of contents and point of view; it will undoubtedly receive the welcome it deserves at the hands of plant physiologists and horticulturists.—C. A. SHULL.

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[7] CHANDLER, W. H., Fruit growing. 8vo. pp. xvi+775. figs. 60. New York: Houghton Mifflin Co. 1925.

Growth of biology

A knowledge of the historical background of science is not only very desirable, but well nigh indispensable to both teachers and investigators. The history of biology, like that of physics and chemistry, is replete with the romance of discovery and achievement. The late Professor LOCY recognized the importance and significance of the history of biological science, and at the time of his death left the manuscript of a book[8] which will be eagerly read by those who have been delighted with his earlier work, *Biology and its makers*. The book should be read by all who desire an understanding of the romantic development of the great ideas, principles, and speculations of biological science and philosophy.

The development of botany and zoölogy runs parallel, and the author treats these sciences together in telling his story. Each has contributed equally to the study of microscopic structure, the investigation of protoplasm, the formulation of the cell theory, and the other generalizations of modern biology.

Students of botanical history will be interested especially in the chapters on the natural history of antiquity, and of the Greek and Roman periods, the story of the herbals of the sixteenth century, the contribution of LINNAEUS, the period from LINNAEUS to SCHLEIDEN, and the period dominated by HOFMEISTER'S work.

The book is written in a charming, entertaining style, of which the author was a master.—C. A. SHULL.

Essentials of systematic pomology

Students of systematic pomology will find much in this book by DRAIN[9] to commend it. It aims to be a textbook for student use, and is a well organized presentation. It is written in clear readable style, and contains much information that will be of value to those who must know the varieties of pomes and other fruits accurately.

There are twenty-one chapters, arranged in such a way that the student makes a careful study of the various fruits, and then is introduced to the classification of the group. The first five chapters deal with the pomes, the second five with the drupaceous fruits, and the following eight with small fruits. The last three chapters consider southern fruits (citrus, olive, pineapple), nut fruits, and nomenclature ot fruits, with the code adopted by the American Pomological Society.

The author makes effective use of his observations and experience with students, with the result that the book is interesting, even to an amateur. It also contains much valuable information for those who may be interested in producing fruit in their own gardens.—C. A. SHULL.

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[8] LOCY, W. A., Growth of biology. 8vo. pp. xiv+481. *figs*. 140. New York: Henry Holt. 1925.

[9] DRAIN, B. D., Essentials of systematic pomology. 8vo. pp. vi+284. New York: John Wiley & Sons. 1925.

NOTES FOR STUDENTS

Mechanism of oxidation.—Several recent papers deal with the oxidase system in plants. GALLAGHER[10] has suggested, not without opposition, that the "oxygenase" of BACH and CHODAT is not necessary to the system. From the potato and mangold roots, he has been able to extract a lipin-like autoxidizable body, which he believes may function as a peroxide former. In this way the production of the organic peroxide of the system would need no catalyst. Many such autoxidizable compounds exist in plants, for the unsaturated terpenes are all able to absorb oxygen. Among these may be mentioned limonene, cumene, terpineol, carvone, etc., fairly common constituents of plant extracts.

The nature of the peroxidase component is considered in a second paper,[11] in which the claim is made that peroxidase activity is associated with aliphatic aldehydes, but atmospheric oxygen is required to produce the peroxidase through formation of some oxide. Substances behaving as peroxidases are generally characterized by the presence of an oxide group of the type $=O$, and GALLAGHER believes that some aldehyde derivative like $R.CH=O=O$ is responsible for the peroxidase activity. The peroxidase of mangolds has been investigated[12] with reference to its thermostability. Although its activity is inhibited by heating, the effect is temporary, reactivation occurring on standing in the cold. The peroxidase is associated with iron, and the opinion is expressed that iron may be the oxygen carrier in the formation of the aldehydic monoperoxide.

GALLAGHER'S position is criticized by ROBINSON,[13] and ONSLOW,[14] who maintain that catechol derivatives form the basis of the organic peroxides of the oxidase system, with two enzymes: (a) oxygenase to catalyze the formation of peroxide from catechol; and (b) peroxidase to catalyze the splitting of the peroxide during oxidation.

The main contention is that there are so many autoxidizable bodies in the plant, that the lecithin-like compounds cannot be singled out for such a function. Formic, acetic, valeric, benzoic, salicylic, and cinnamic aldehydes, caprylic alcohol, oleic and pyruvic acids, many terpenes, and most plant extracts show autoxidation.

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[10] GALLAGHER, P. H., The oxygenase of BACH and CHODAT; function of the lecithins in respiration. Biochem. Jour. **17**:515–529. 1923.

[11] ———, An investigation of substances capable of behaving as peroxidases. Biochem. Jour. **18**:29–38. 1924.

[12] ———, Observations on the thermostability of the peroxidase of the mangold. Biochem. Jour. **18**:39–46. 1924.

[13] ROBINSON, MURIEL ELAINE, A comparison of certain oxidizing enzymes of the higher and lower plants. Biochem. Jour. **18**:543–548. 1924.

[14] ONSLOW, MURIEL WHELDALE, The oxygenase of the higher plants. Biochem. Jour. **18**:549. 1924.

GALLAGHER's reference to iron in connection with peroxidase is of interest. The influence of iron in the respiratory process has received attention by animal physiologists, but not so much by plant physiologists. In a recent lecture, WARBURG[15] takes the position that iron is the only oxidizing agent in the living cell. He offers much interesting evidence for his view, which, even if not conclusive, should stimulate plant physiologists to study the relation of iron to these oxidase systems which have been proposed. Iron has not been excluded from the oxidase preparations as made by ONSLOW and others, and WARBURG's conception of the rôle of iron in respiration may have wide applicability in cellular oxidations of plants and animals.—C. A. SHULL.

Taxonomic notes.—YAMAMOTO,[16] in his first supplement to Icones Plantarum Formosanarum, publishes the results of collections of new material in four families. In addition to species already published and critical notes, 19 new species are described, one in Moraceae, 10 in Urticaceae (6 of them *Pilea*), 7 in Aquifoliaceae (all *Ilex*), and one in Convolvulaceae.

ROBINSON,[17] in continuation of his study of the Eupatorieae, has some new material from Mexico, including 10 new species of *Eupatorium*.

MUNZ and JOHNSTON[18] have published an account of 12 species of *Oenothera* from South America, 3 of which are new.

JOHNSTON[19] has published descriptions of 23 new species of American plants, the largest contribution being 16 new species in *Fuchsia*.

VAN SLOOTEN[20] has published a very detailed revision of the Flacourtiaceae of the Dutch East Indies. It includes 16 genera, 3 of which (*Eleutherandra*, *Hemiscolopia*, *Mesaulosperma*) are new, representing 75 species, 10 of which are new. The largest genus is *Casearia*, with 18 species.

NAKAI[21] has described two new genera of Bambusaceae, segregates from *Arundinaria*. *Pleioblastus* includes 7 species from China and Japan, while *Indocalamus* includes 7 species from China, India, and the Philippines.

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[15] WARBURG, O., Iron, the oxygen carrier of respiration-ferment. Science N.S. **61**:576–582. 1925.

[16] YAMAMOTO, YOSHIMATSU, Supplementa Iconum Plantarum Formosanarum I. pp. 47. 1925.

[17] ROBINSON, B. L., Records preliminary to a general treatment of the Eupatorieae. V. Contrib. Gray Herb. N.S. no. 75. pp. 1–15. 1925.

[18] MUNZ, P. A., and JOHNSTON, I. M., The Oenotheras of northwestern South America. Contrib. Gray Herb. N.S. no. 75. pp. 15–23. 1925.

[19] JOHNSTON, I. M., Some undescribed American Spermatophytes. Contrib. Gray Herb. N.S. no. 75. pp. 27–40. 1925.

[20] VAN SLOOTEN, D. F., The Flacourtiaceae of the Dutch East Indies. Bull. Jard. Bot. Buitenzorg III. **7**:291–421. 1925.

[21] NAKAI, T., Two new genera of Bambusaceae. Jour. Arnold Arboretum **6**:145–153. 1925.

REHDER[22] has described a new bigeneric hybrid, discovered by JACK in Idaho on the summit of Elk Butte. The parents of the hybrid are said to be *Sorbus sitchensis* and *Amelanchier florida.* While several bigeneric hybrids are known among the Pomoideae of Rosaceae, the present hybrid (*Amelasorbus*) is of interest because the two genera producing it are much less closely related than those producing the previously known hybrids.—J. M. C.

Ecological aspects of pathology.—That important pathological problems may be ecological in part is well illustrated by a recent study of the western yellow blight of the tomato by SHAPOVALOV.[23] It is shown that the occurrence of this disease is decidedly affected by weather conditions, the general fact being that the spread of the blight is favored by conditions that raise the rate of evaporation, and opposed by an increase in atmospheric humidity. The exact manner in which evaporation facilitates the development of the blight is not clear. Perhaps the fungus attack may be a secondary phenomenon following the abnormal development of the plant during periods of high evaporation.—H. C. COWLES

Bog xeropytes.—The old and unsettled problem of the real character of bog plants has recently had suggested a solution that appears plausible, and is certainly very suggestive and stimulating. Studying in the field the heath and bog plants of northwestern Germany, STOCKER[24] concludes that SCHIMPER's theory of bog xerophytism is incorrect. Measuring the rate of transpiration of such plants, he finds that it is comparable with that of plants growing in other habitats; in fact, when the criterion of the ratio of daily transpiration to weight of roots is adopted, the heath and bog plants have a very high rate of water loss.

STOCKER also claims that the heather, *Calluna vulgaris,* is able to thrive only where atmospheric humidity is high, and in regions where there are dry summers only where it is protected by trees whose shade makes locally humid conditions. The xeromorphic character of the leaves of these ericaceous heath and bog plants are regarded as adaptations, to protect these plants from the high winds of winter storms that prevail in the regions of heaths and bogs. It is suggested that as these leaf forms protect against injury by high winds rather than against water loss, they be termed "anemomorphs" rather than "xeromorphs."—GEO. D. FULLER.

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[22] REHDER, ALFRED, *Amelasorbus*, a new bigeneric hybrid. Jour. Arnold Arboretum **6**: 154–156. 1925.

[23] SHAPOVALOV, M., Ecological aspects of a pathological problem (western yellow blight of tomatoes). Ecology **6**: 241–259. 1925.

[24] STOCKER, O., Die Transpiration und Wasserökologie nordwest deutscher Heide- und Moorpflanzen am Standort. Zeitschr. 1923.

———, Klimamessungen auf kleinstem Raum an Wiesen-, Wald-, und Heide, pflanzen. Ber. Deutsch. Bot. Gesells. **41**: 145–150. 1923.

———, Ökologisch-pflanzengeographische Untersuchungen an Heide-, Moor- und Salzpflanzen. Die Naturwissenschaften **12**: 637–646. 1924.

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STUDIES IN THE GENUS XANTHIUM

JENNIE L. SYMONS

(WITH PLATES IX–XI)

Introduction

The studies in the genus *Xanthium*, of which this is a preliminary account, were begun in the autumn of 1922. An attempt was made to obtain seeds from all parts of North America, thus to gain some idea of the distribution of species. It was hoped, also, that a study of the plants, from germination to maturity, would throw some new light on the taxonomy by revealing genetic relationships in the genus. An experimental garden has been devoted exclusively to *Xanthium* for two summers. In 1923, seeds from 22 different sources in North America were grown. In 1924, all the seeds planted had been collected in two small areas on opposite shores of the St. Lawrence River at Montreal. Small numbers of plants have been grown in the McGill University greenhouse during the winter months of both years. Observations have been made upon the main differences in habit, foliage, relative time of flowering, and other points not easily determined from herbarium material. Breeding experiments have been carried on, particularly on the St. Lawrence River forms. As a check upon the results thus obtained, some cytological studies and chromosome counts have been made. Incidentally, notes have been taken during both summers upon the occurrence of delayed germination in the garden.

In the beginning of the investigation, letters were sent to the Departments of Agriculture in Washington and in Ottawa, and eventu-

ally to all the Agricultural Experiment Stations in the United States and Canada. The response to these letters was very generous. Burrs were received from 22 different sources in North America, and from 4 different parts of Europe. When burrs were still attached to the plants in the field, trouble was taken to wrap fruits from single plants in separate packages, in accordance with my request.

Several collecting trips were made to the St. Lawrence River shores, at Point aux Trembles on the north side and at Longueuil on the south.

Plants were taken in the field, numbered, wrapped separately in newspaper packages, and brought to the laboratory. There they were stripped of their burrs, which were kept in glass jars until spring. The same collecting grounds were visited again in May, when the seedling plants had just unfolded their cotyledons; 107 of these small plants were taken up and successfully transplanted into the experimental garden. On the same collecting trips large masses of detached burrs were found mixed with rope waste and other material which had been washed on shore when the river was at its highest level. Numbers of these were gathered for planting in the garden.

Method

Plantings were made 2 feet apart. The rows were 2.5 feet apart as a rule. The location of each seed was marked by a wooden label on which the source of the seed and the date of planting were noted. Fruits which had been classified as belonging to the same species were planted in the same part of the garden. In the first season, fruits and seeds were planted from each lot received. In each case, half the burrs were planted whole and half were opened, the seeds then being planted separately. In the second season, fruits and seeds from 42 different plants were sown, as well as the detached burrs collected in the spring. The seedlings were planted first, 2.5 feet apart, in the center of the garden. In both seasons the planting was done during the first week in June.

When the buds first appeared, plants were selected for breeding experiments. Staminate and pistillate flowers being separate in *Xanthium*, pollination is easily controlled. Staminate heads were removed with forceps which were sterilized by dipping in absolute

alcohol. Several plants of each type grown were thus emasculated and bagged before the stigmas had appeared in the female blossoms. Others were bagged to insure purity of pollen for the controlled pollination. Between four and five hundred branches were thus protected. The bags were of the usual square-bottomed sort, of strong brown paper, in medium size. To facilitate pollination, the bottom of the bag was cut around three sides and made fast again with paper clips. The blossoms could thus be observed from time to time and treated when sufficiently developed. In transferring pollen from one plant to another, it was found most practicable to cut the male branch and carry it, still inclosed in its bag, to the branch to be pollinated. There, both bags were carefully opened and the pollen shaken in upon the exposed stigmas. Many crosses were tried, with all possible combinations of species. Some branches of each kind were left for self-pollination. In each species some branches which had been emasculated were left unpollinated to test for apogamy. In a few cases, pollen was shaken up with a little pure water and applied with the finger-tip, a method which also succeeded. After pollination, the clips were replaced and the bags left until late September. After a heavy rain, it was usually necessary to loosen the cord which tied the bags at the base of the branch to let out any water which had accumulated. A few branches being attacked by fungi in September, when the fruits were well developed, the bags were removed. Fruits were gathered in late October.

For the cytological work, staminate heads were collected when young from all species and varieties in the garden. The fixing fluid was taken into the field in small bottles which were labeled as the material was gathered. A modification of Bouin's fluid was used for fixation. It was made up according to the directions of ALLEN (1) as follows:

Picric acid (sat. aq. sol.)	75 cc.	
Formol	25 cc.	
Glacial acetic acid	5 cc.	
Chromic acid crystals	1.5 gm.	Dissolved in the fluid just before use
Urea crystals	2.0 gm.	

After 1–3 hours in the fixing fluid, the material was washed in 75 per cent alcohol until no yellow color remained. It was preserved in

70 per cent alcohol until imbedded in paraffin. The sections were stained with iron-alum haematoxylin. The best results were obtained when slides were left in the haematoxylin at least 24 hours.

For the fixation of material grown in the greenhouse Carnoy's fluid was used. This fixative was as satisfactory with *Xanthium* material as the more elaborate one just described. The smear method, as recently outlined by TAYLOR (22), was also tried with some success.

Investigation

I. TAXONOMY AND GENETICS

In the first season, as the plants grew, the variation in habit and foliage was greater than had been expected. With a few exceptions, the habit of plants from different sources differed enough to enable even a casual observer to distinguish readily the point in any row where plants from one locality adjoined those from another.

None of the southern forms blossomed in time to produce any fruits, although the plants were large and healthy. Plants from farther north grew less luxuriantly, but matured abundance of fruit. The burrs were similar to the ones which had been planted in each case, although in some instances it was difficult to identify them with certainty from the available classifications. Eventually, specimens of all these forms were taken to Washington for comparison with material in the National Herbarium. In many cases, conclusions already reached were confirmed, but some uncertainties remained. The greatest difficulty was with forms collected on the banks of the St. Lawrence River, near Montreal. MILLSPAUGH and SHERFF (11), in their first paper on *Xanthium*, state the condition of affairs as they found it as follows:

> In determining certain specimens of *Xanthium* in the Herbarium of the Field Museum, the writers have found the taxonomic status of this genus, as concerns its various species, to be very unsatisfactory at the present time. Much of the uncertainty in connection with several species arises from the difficulty encountered in the past in identifying the older specific names, names that in a number of cases, at least, were founded upon heterogeneous material. . . . Another source of confusion in herbaria has been the erroneous identification of new and undescribed species with any one of the older, more commonly known species. Still further, we must note the well-known contempt with which common weeds such as *Xanthium* are so often regarded, a reason that explains the

surprisingly small number of herbarium specimens of any one species collected heretofore in a given region.

Later, in their monograph on North American species of *Xanthium*, in which they thoroughly review the taxonomic literature of the genus and greatly reduce the number of problems in bibliography, they point out the less easily solved difficulty as to species concept.

> The species concept in *Xanthium* . . . . must long remain a perplexing problem. In temperate regions the plants do not mature their fruits sufficiently for exact determination until after frost comes and the majority of collecting botanists have ceased their field work. This renders good material in herbaria scanty in quantity and inadequate in quality. Some of the species are known to exhibit most striking variations in fruiting characters, variations that with many botanists would be taken to represent varieties or subspecies. . . . . We have retained several of the less well-known species because we have felt that only after further field observations and breeding tests can satisfactory conclusions as to their true status be reached.

Some forms still defying classification, it seemed futile to describe them as new species until their purity was established. This certainty as to purity of species seemed also necessarily to precede any real conclusions about their distribution. It was therefore decided to devote the summer of 1924 to breeding experiments upon a limited number of forms. Those collected on the shores of the St. Lawrence River at Montreal were chosen. There were several reasons for this choice. First, abundance of material was available and field observations could be made at different seasons as a check upon experimental results. Second, some of the most interesting and baffling of the forms thus far observed had been collected in this locality. It seemed highly probable, owing to the action of the river as a distributing agent, that a greater number of species would be found in a small area here than in most places. If natural conditions permitted hybridization in the field, many hybrid forms would also be found.

LASCH (6), as early as 1856, finding in one locality three species of *Xanthium* and several forms which combined certain of their characteristics, described the latter as hybrids. BITTER (2), however, was the first to do actual breeding experiments with species of *Xanthium*. He reported, in 1908, that he had successfully crossed *X. macrocarpum* DC. and *X. italicum* Mor. with *X. strumarium* L. His cultural studies had led him to the conclusion that within the

section Euxanthium hybridization could take place freely, although crosses could not be made between any of these species and *X. spinosum* of the section Acanthoxanthium.

SHULL (17) found that the three main types of *Xanthium* which commonly occur in the vicinity of Lawrence, Kansas, do not hybridize in the field to any extent because they blossom at different times. "There is a physiological isolation that effectually prevents hybridization in the great majority of cases. The pollen of one variety has been shed long before the stigmas of the other are ready for the pollination processes." Whether such physiological isolation occurs in other localities must be tested in each case.

From the fruits of 42 Montreal specimens, 471 plants were obtained; 250 additional plants developed from the detached burrs which had been collected in the spring. The 107 transplanted seedlings made a total of 828 plants in the garden in the season of 1924. The probability was that these would include all forms of *Xanthium* common in this locality. The first buds appeared early in August on the plants raised from seedlings. In the third week in August, there were blossoms on practically all the plants. There seemed to be no such isolation here as SHULL had found in Kansas.

The recent classification of MILLSPAUGH and SHERFF (11) was used in identifying species. Even with the help of the full discussions and clear plates in this monograph, as well as the original description of each species, some difficulties remained. The form most common on the south shore of the St. Lawrence River was identified as *X. italicum* Moretti. In the key of MILLSPAUGH and SHERFF, *X. echinatum* Murr. and *X. italicum* Mor. are separated by the relation between the length and breadth of the body of the fruit. As the body of the burrs in question was invariably more than twice as long as wide, this point was easily decided. A study of the plates, however, led to the conclusion that the burrs were more like *X. echinatum* in appearance. Moreover, one of the burrs pictured as *X. echinatum* was collected by VICTORIN at Longueuil, where the writer's specimens were gathered. The photograph of this fruit was measured and found to be 17 mm. in length, and 7.5 mm. in breadth, surely more than twice as long as wide. A critical study of the descriptions of *X. echinatum* Murr. and *X. italicum* Mor. as given by MILLSPAUGH

and SHERFF, shows them to be alike in many ways and distinguishable chiefly by measurements of the mature fruit. The leaves and stems of *X. italicum* may attain a considerably greater length than those of *X. echinatum*, but a small plant of the former could not be distinguished in this way. Both have scabrous, purple-spotted

TABLE I

	Length (mm.)			Breadth (mm.)
	Beaks	Prickles	Body	Body
Average (125)	5.1	4.3	15.9	7.2
Range of variation (125)	4.1–5.9	3.4–4.8	14–18	6.5–8.0
Range X. echinatum	3–5	3–5	16–20	8–10
Range X. italicum	5–7	3–7	13–18	6–8

stems, scabrous leaves which may vary considerably in form, and burrs with body and prickles bearing glands and hairs, and beaks at least partly hispid. Both are reported from Quebec. The beaks of both are described as incurved, although in the case of *X. italicum* the limiting word "plerumque" allows the possibility of their being

TABLE II

	Stem (dm.)	Length of leaves +petioles (dm.)
Range in form studied	6–10	1.3–3.5
Range in X. echinatum	3–6	0.6–2.3*
Range in X. italicum	3–10 (–18)	0.8–3.0*

* Petioles only, about equal to the leaf blades or exceeding these.

straight. In the original description of *X. italicum* (13) the beaks are said to be "linear." The beaks of the writer's Longueuil specimens were sometimes divergent and sometimes incurved.

Measurements were taken of 125 burrs of the species in question. The result is shown in table I, together with the range of measurement given in the descriptions of the two similar species.

Further measurements were taken of stems and leaves (including petioles) and compared as shown in table II. The measurements are seen to conform generally to those expected for *X. italicum* Mor.

Another point omitted from the original descriptions (13, 14), as well as from the formal descriptions by MILLSPAUGH and SHERFF, is suggested by the discussion of these authors with regard to *X. maculatum* Rafinesque, which they reduce to synonymy with *X. echinatum* Murr. They quote part of RAFINESQUE's description, emphasizing in italics the points which lead them to believe that he was describing *X. echinatum.* One of these emphasized phrases is "generally solitary." The fruits of the species occurring commonly at Longueuil are never solitary, so far as the writer has observed, but occur in thick clusters. A similar difficulty was encountered in distinguishing *X. inflexum* Mack. and Bush (7) from *X. pennsylvanicum* Wallr. Both species apparently occur sparingly here, and may have hybridized to some extent with recombinations of hereditary units. Table III shows the measurement range given for the two species by MILLSPAUGH and SHERFF.

TABLE III

	Length (mm.)			Breadth (mm.)
	Beaks	Prickles	Body	Body
X. pennsylvanicum........	4–6	3–7	10–20	5–8
X. inflexum..............	5–7	4.5–6.5 (8–10 near beak)	13–17 (–20)	6–7.5

It will be noted that these measurements overlap to a great extent, and unless there are other distinctive characters, there will often be doubtful cases where the two forms occur together. The original description of *X. inflexum* speaks of the beaks as "about 10 mm. long, at maturity abruptly bent at the middle, inflexed and at length overlapping, hooked at the apex." MILLSPAUGH and SHERFF embody all these points in their description, but disagree as to the length of beaks. The beaks of *X. pennsylvanicum* are somewhat variable, according to the descriptions, as to thickness and form. According to MILLSPAUGH and SHERFF, *X. inflexum* is a taller plant than *X. pennsylvanicum,* the former ranging from 10 to 15 dm. in height, the latter from 3 to 9 dm. If the original descriptions are consulted, there is also a difference in the tips of the prickles. WALLROTH (23) describes those of *X. pennsylvanicum* as "ending in a short,

lightly bent hook," whereas MACKENZIE and BUSH speak of the prickles of *X. inflexum* as "strongly hooked." These writers do not mention the arcuate character of the spines, which, however, is pointed out by MILLSPAUGH and SHERFF, and which is not a character of *X. pennsylvanicum*. These seem to be the main differences.

The species agree in general stem and leaf characters, and, to a great extent, in the size and shape of the fruit. Both are comparatively smooth as to body and prickles, which bear glands, but few, if any, non-glandular hairs, except at the base of the beaks. Table IV shows the result of measurements of 96 burrs from 8 plants classified as *X. pennsylvanicum*.

TABLE IV

	LENGTH (MM.)			BREADTH (MM.)
	Beaks	Prickles	Body	Body
Average (96)......................	6.1	4.9	18.0	7.0
Range (96).......................	4.5–9	3.5–7	13–22	5.5–9.5
Range X. pennsylvanicum.........	4–6	3–7	10–20	5–8

The burr which showed a body thickness of 9.5 mm. was not an exceptional fruit, but grew upon a plant with broadly ovate burrs with an average thickness of 8 mm., few measuring less than 7 mm. thick. This, of course, agrees more closely with *X. pennsylvanicum* than with *X. inflexum*. The prickles, however, were very strongly hooked and arcuate. They ranged in length between 3.5 and 5.5 mm.[1] The beaks in some cases were bent inward from the middle. They measured 5–6 mm. in length. The body length varied between 18 and 20 mm. The body and base of the prickles were thickly dotted with glands. There were few hairs and these almost entirely on the lower part of the beaks. The height of the plants was 7.5–10 dm. These characters were common to the original mother plant and to those grown from its fruits. It will be noted that the arcuate, strongly hooked character of the prickles is typical of *X. inflexum*. The plants also are comparatively tall. This might easily be due to cultivation, but it will be remembered that *X. italicum* did not reach its maxi-

[1] In the discussions, averages concerning progeny of individual plants were usually obtained from measurements of 10 fruits chosen at random.

mum height under the same conditions. The most striking misfit in the table is the beak measurement of 9 mm. This burr was on a plant which had fruits with an average beak length of 7.3 mm., far exceeding normal *X. pennsylvanicum* measurements. The beaks, in some cases, were strongly inflexed. There were instances, also, of the *inflexum* habit of much longer spines near the beaks. These spines, however, did not exceed 7 mm. All the other characters were typical of *X. pennsylvanicum*, and it was therefore classified, for the time, as belonging to the latter species. These two cases, among others noted, seem to support the theory that there has been hybridization, with recombination of characters.

TABLE V

	Length (mm.)			Breadth (mm.)
	Beak	Prickles	Body	Body
Average (40)	6.3	5.6	17.6	6.9
Range (40)	5–9	4.5–7.5	15.5–22	5.5–8.0
Range X. inflexum	5–7 (–10)	4.5–6.5	13–17 (–20)	6–7.5

Table V shows the average and the range found in measuring 40 fruits from 4 plants considered to be *X. inflexum* Mack. & Bush. The beak length of 9 mm. is not too long according to the original description. Evidently the beaks can at least occasionally attain this length.

It has been mentioned in all descriptions of *X. inflexum* that there are occasional longer spines near the beaks. In this species, the average length obtained for prickles depends, therefore, on which ones are measured; 8 mm. is wide for *X. inflexum*, but the original description states only that the body is "2 cm. or less long and less than half that in width." If the proportion is maintained, as it was in all cases observed, the conformity is probably sufficiently close. The other characters of these plants seemed typical of *X. inflexum*. The height of the plants, however, rarely exceeded one meter, and in some cases fell to 6 dm.

The most difficult problem in classification of an apparently pure species was furnished by certain plants collected on the north shore of the St. Lawrence River. The fruits bore a striking resemblance to

those figured by MILLSPAUGH and SHERFF (12) as *X. curvescens*, sp. nov. They had the same stout, divergent, strongly arcuate and hooked beaks, as well as the comparatively few curved and hooked prickles. They were thicker, however, and of a light brown color. While not very hairy, the prickles and beaks were sparsely hispid to about the middle. Among the hairs, as well as distributed over the surface of the body, were numerous glands. The plants, on the other hand, did not at all fit the description in vegetative characters. The stem, far from being "reddish, scabrous, about 3 dm. high," was comparatively smooth and green, usually with green lenticels; the latter, in a few cases, became purple. The plants were tall (9–12 dm.) and grew luxuriantly. Leaves (including petioles) ranged from 1.7 to 3 dm. in length. The blades were larger, thin, and of a light green color, distinct from all the other plants grown. This species was called *X. curvescens*, type *A*. A closely related form was designated type *B*. It had the same general characters as type *A*, but its fruits were less coarse in appearance; the beaks and prickles were shorter, the latter being about twice as numerous. The leaves were firm and of a distinctly darker green. Body and prickles were almost glabrous, although both bore glands. The contrast in foliage between these two types was so striking that fresh leaves were gathered from each, in order to determine, if possible, the reason for the difference in color. An account of this experiment will be given in another section.

Only one plant of type *C* was found. It differed from type *B* chiefly in the hispid character of its body, beaks, and prickles. Its body also had a greater length in proportion to its breadth. Two other plants, of the same general type, had fruits which more nearly fitted the description of *X. curvescens* in regard to their measurements. When these fruits were opened, however, very few seeds were found. This suggested the possibility of type *D*, *X. curvescens*, having arisen apogamously from types *A* or *B*, in which case it would be a haploid form. This point could best be determined by chromosome count, of course, and an attempt was therefore made to obtain material for this. The fact that so many varieties of one species were found supports BITTER's opinion that the Euxanthia, like the North American species of *Oenothera* of the *biennis* group, are now in a state of intensive separation. Ninety-five fruits of this species, including

types *A*, *B*, and *C*, were carefully measured. Table VI shows the range of variation in size for each type, in comparison with that described for *X. curvescens* MILLSPAUGH and SHERFF. The question arises as to whether this species may be *X. orientale* L. (*X. macrocarpum* DC.).

MILLSPAUGH and SHERFF, in their monograph, show that *X. macrocarpum* DC. was merely a new name created with reason by DE CANDOLLE for *X. orientale* Linn. As a result of their study of the

TABLE VI

MATERIAL	LENGTH (MM.)			BREADTH (MM.)
	Beaks	Prickles	Body	Body
Type A (measurements from 45 fruits from 4 plants)				
Average	5.4	4.6	15.9	6.5
Range	4–7.5	3–7	13–21	5–8.5
Type B (40 fruits from 4 plants)				
Average	4.6	4.0	16.4	6.4
Range	3.5–6.5	3–5	13.5–21.0	5–9
Type C (10 fruits from 1 plant)				
Average	4.9	4.1	17.6	5.9
Range	4–6	3.5–5	16–21	5.5–7
X. curvescens M. & S.	....	3–6	13–16	3.5–5

species and the literature concerning it, these authorities say, "The true *X. macrocarpum* DC. is a plant with strongly hooked beaks and the prickles somewhat subremote, stoutish, tending to be not only hooked at the apex but also arcuate, often backwardly, then forwardly, from about the middle upward." Discussing specimens of *X. orientale* examined from Europe in comparison with *X. curvescens* from Vermont, they state that in the former they "have found the fruiting involucres to be not only considerably larger but brownish rather than reddish, also much more pubescent and the prickles nearly always more numerous." All this would apply to fruits of the different types of the St. Lawrence River species, and it seems probable that the European species has become established here.

Before any planting had been done, it was thought that *X. Wootoni* Cockerell (ex DE VRIES 5) occurred here. Four plants in the field which bore typical *X. italicum* fruits also bore fruits which would certainly have been classified as *X. Wootoni* if they had been

found as detached burrs (pl. I, *A 1*). No plants were found which had only *X. Wootoni* fruits, however, and the *X. Wootoni* burrs planted here produced only the parent type, with occasional fruits like themselves. RYDBERG, of the New York Botanical Garden, kindly allowed the writer to copy a letter written in 1902 from COCKERELL to himself concerning this species. COCKERELL, who discovered it in New Mexico, first found the plants distinct. Later he was somewhat puzzled when he found "on a plant at Las Vegas, a burr of each sort, that is, of *X. Wootoni*, and what we call *X. canadense*, the latter with very crowded prickles." This specimen was regarded as exceptional at the time and placed in the herbarium at Misella Park. Hundreds of both forms, apparently pure, were growing in the same locality. DEVRIES later planted burrs of both kinds sent to him by COCKERELL, and found that they produced plants true to type. In 1913, COCKERELL (3) found in a greenhouse at Boulder a specimen of *X. commune* (now called *X. italicum*) having several *X. Wootoni*-like burrs, although no *X. Wootoni* had ever been seen in Colorado. These duplex specimens reported by COCKERELL seem to have been like the ones found at Montreal. *X. Wootoni* may yet be found here, although the specimens thus far observed seem to be merely varying forms of *X. italicum*. The frequent occurrence of a similar variation under the artificial conditions of the greenhouse, probably comparable with the Boulder specimens, will be discussed later.

Another very striking form, which had fruits varying constantly between two extremes for two generations, has defied the writer's attempts to classify it. The fruits originally collected were fairly uniform in size. Their measurements, as well as body and beak characters, were typical of *X. italicum* Mor. The prickles, however, were generally arcuate and varied greatly in number. The fruits, stripped off and sorted into classes, showed all grades of transition between *X. italicum* and *X. Wootoni*, while many of the prickles were strongly arcuate. The plants grown from these burrs were all alike, and distinctly different in appearance from any other variety in the garden. They were erect and very short, averaging 5–6 dm. The stem had the vertical purple lines characteristic of so many species. The leaves were rather small, thick, and coarsely serrate, a dull, dark green in

color. All plants produced fruits having the same range of variation as the parent, burrs with many and few prickles occurring in the same cluster.

There was a marked difference between the two forms just described, although both were apparently varieties of *X. italicum*, and both produced some burrs of the *X. Wootoni* type. In the one case, there were only the two kinds of burr, as there seem to have been in the specimen found by COCKERELL. In the other, the number of prickles on the fruits varied very greatly, forming a series between the two extremes. The latter differed also in vegetative characters from typical *X. italicum*, and seemed to be an ever-sporting variety.

Another striking case was that of a plant which bore burrs of the two most distinct types of *X. curvescens*, *A* and *B* (pl. IX, *A3*). They were alike in having only glandular hairs upon body and prickles, and in the arcuate character of their spines and beaks. The coarser type, however, had less than half as many prickles as the other. It was lighter in color and thicker in diameter. Twelve fruits from this plant were sown in the garden and produced fruits of one type, the vegetative characters being those of type *B*. The number of prickles and other fruit characters seemed to be intermediate between the two.

Other types grown by the writer behaved like hybrids, splitting up in the F_2 generation into the parent forms. Mendelian ratios were not obtained exactly, as comparatively few plants were grown from any one parent. For example, 12 fruits from a plant which had all the characteristics of *X. italicum* Mor. produced descendants of two types, about half with burrs of the *X. inflexum* type and the others with fruits having hispid prickles and other characters of *X. italicum*. A second example of what seemed a natural hybrid was furnished by a row of 14 plants, grown from 8 burrs from the same plant of typical *X. pennsylvanicum* character. In the next generation the plants were tall and erect, with smooth green stems and firm scabrous leaves. Some of them, however, bore fruits resembling *X. chinense*, while others had fruits like those of the parent observed. Plants with burrs of the *X. chinense* type were more numerous. Their fruits were small, with few prickles, and characteristic wide open beaks, which were purple-tipped when immature. The latter, usually indicative of the

number of seeds in the burr, varied in number from 2 to 7. The formation of more than the normal number of beaks and seeds was especially characteristic of these *X. chinense* plants, although it occurred to some extent in all plants of this lot. In one case a specimen of each sort came from a single burr.

These cases are suggestive, but it is clear that without controlled pollination Mendelian results can hardly be expected in a locality where several species, between which hybridization is possible, grow and blossom at about the same time. If two forms have crossed in the field, there may be inbreeding in the F_1 generation, but it is possible that pollen from another species or variety will reach at least some of the stigmas. In such a locality as the one here dealt with, where masses of mixed burrs are washed ashore in early spring and germinate close together, many varieties can sometimes be collected within a few yards. It is probable, therefore, that in such spots polyhybrids arise, and that the ovules of a single plant will sometimes be fertilized by pollen from more than one source.

The results of the controlled breeding experiments are summarized as follows. About half of the attempted crosses were successful. In most instances the same cross which failed on one branch succeeded on another. In these cases at least, therefore, the failure was not due to a physiological incompatibility between the species or varieties. The experiments took considerable time. In some cases the pollen had been formed long before it was applied, and in other cases the stigmas may have been too old before pollen was placed upon them. Good seeds were set by all species when the plants were self-pollinated. The following crosses were successful:

1. *X. italicum* crossed with *X. inflexum*
2. *X. italicum* crossed with *X. curvescens A*
3. *X. italicum* crossed with *X. curvescens B*
4. *X. curvescens A* crossed with *X. curvescens B*
5. *X. curvescens B* crossed with *X. inflexum*
6. *X. curvescens B* crossed with *X. pennsylvanicum*

It will be noted that *X. pennsylvanicum* was crossed in only one case. Unfortunately it was found when the fruits matured that this plant of *X. pennsylvanicum* had been heterozygous. The apparent isolation here, however, is not thought to be real. Few crosses were

attempted with this species, the reason being that there was a relatively small number of *X. pennsylvanicum* plants, and few of these were reached while in bud during the time of emasculation and bagging.

Out of more than 50 branches emasculated and left unpollinated, only two were found to have formed burrs to any extent. One of these was of the *X. curvescens A* type. There were 22 apparently normal fruits. These were opened and contained 8 seeds altogether. The other was on one of the heterozygous plants of *X. pennsylvanicum.* Many burrs were opened, but only 9 seeds were obtained. Since there were many branches of each of these types which had been treated similarly and had produced no seeds, it is highly probable that in these two cases a small staminate head had been overlooked in the emasculation, or that a new one had developed later and pollinated these ovules. There were three other instances in which a single seed was found among many fruits opened.

A few of each kind of seed obtained in the breeding experiments were planted in the greenhouse in January, 1925. The main object at that time was to obtain blossoms for cytological study and chromosome count. Owing to lack of space, it was impossible to get any numerical results for genetic conclusions. The burrs were allowed to develop, however, and it was hoped that from them some conclusions might be reached with regard to the heredity of the plants studied. The seeds were planted about 9 in. apart, in boxes 10 in. deep. The house in which they were grown was kept at a temperature of 76° F. during the day. The outdoor temperature was very low at this period, and the temperature inside fell many degrees during the night. The glass had been coated with white lead and no direct sunlight came in. Germination was slow and the seedlings were weak. It was decided, therefore, to try artificial illumination. Thirty-six 75-watt lamps were attached to a frame which was suspended 2.5 feet above the boxes in which the *Xanthium* seedlings were growing. These were kept burning night and day for about two weeks. The plants straightened up and grew more rapidly. A few were injured, however, by an excess of heat. About March 1 the paint was removed from the glass above the plants, and artificial light was no longer used. Buds appeared soon afterwards, and staminate

heads were collected and prepared for cytological study. The fruits are not yet mature (May 8), but the oldest burrs on each plant have reached full size. Some striking abnormalities have appeared. A summary of greenhouse results follows.

X. italicum Mor. (pl. IX, *B1*).—Burrs from seven plants, identified when collected as *X. italicum* Mor., were grown for two generations, the second from seed of plants self-pollinated in the first. In all these cases the mother plants seem to have been homozygous, all the plants obtained showing *X. italicum* characters.

X. pennsylvanicum Wallr. (pl. IX, *B2*).—Two plants of *X. pennsylvanicum* produced burrs of the same type for two generations, although a few fruits with about half the number of prickles were mingled with typical ones on the greenhouse plants.

X. inflexum Mack. & Bush. (pl. IX, *B3*).—Descendants of two plants of *X. inflexum* were observed for two generations. By the first, *X. inflexum* characters were produced for the most part in both, although the beaks were not bent at the middle in the characteristic way on the greenhouse plants. The other produced *X. italicum* in the second generation, which seemed to indicate that the original plant had been heterozygous.

X. curvescens, type *A* (pl. IX, *B4a*).—Burrs from four plants, identified when collected as *X. curvescens*, type *A*, were grown for two generations in the same way. The first summer all the plants produced fruits of the same kind as those on the parent plant. In the generation grown in the greenhouse, the habit of the plants was of the same nature, and the general shape and size of fruits were the same, but the very striking arcuate character of both prickles and beaks seemed to have been lost.

X. curvescens, type *B* (pl. IX, *B4b*).—Burrs from three plants of *X. curvescens*, type *B*, reproduced the original characters when grown in the garden. Only one of these, selfed and grown in the greenhouse, produced characteristic fruits with incurved beaks and many arcuate prickles. On the same plant were a few abnormal burrs of the *X. Wootoni* type. A second, selfed, produced burrs which fitted the description of *X. pennsylvanicum*. Fruits of the third resembled the original type except in their beaks, which were short, stout, and not incurved.

X. curvescens, type *C* (pl. IX, *B4c*).—Type *C*, selfed, produced only fruits of the abnormal type, with very few prickles and these not curved. The shape and pubescent character of the fruit, however, and the character of the beaks were true to type.

X. curvescens, type *D* (pl. IX, *B4d, e*).—There were very few seeds from the two plants of type *D*. Six were planted and two germinated. Pollen was transferred from one to the other in both directions, and a few burrs were formed on each. In one case they were true to type, in the other a new type appeared with exceedingly long spines and no good seeds. One seed from the former plant was sown in the greenhouse but did not germinate.

HYBRIDS.—Two seeds of each hybrid artificially produced were sown in the greenhouse. Pl. X shows typical specimens. From the genetic viewpoint only very general conclusions can be drawn from the greenhouse results. So far as normal fruits were obtained, it seemed that *X. italicum* qualities were dominant to those of *X. inflexum*, *X. pennsylvanicum*, or any of the types called *X. curvescens*. *A* and *B curvescens* crossed gave fruits of the *A* type. Unfortunately, the plants obtained by crossing *X. curvescens* with *X. pennsylvanicum* died in the seedling stage.

On nearly all the greenhouse plants there were some abnormal burrs, of which the plates show good examples. In every species grown there was occasional reduction in number of prickles, giving rise to fruits of the *X. Wootoni* type. It seemed possible that bud-sporting was common in the genus, and that the tendency was emphasized under the artificial conditions in the greenhouse. A case of fasciation which occurred on a plant of *X. italicum* in the garden, affecting one of the two lowest branches, suggested this explanation. It was found, however, that the abnormal burrs were often scattered here and there in clusters of normal fruits. The fact that the staminate heads had been removed from the tips of the flowering branches suggested that the wounding of the tissue had induced the formation of abnormal burrs. There were clusters on some plants, however, which had not been disturbed and which still bore *X. Wootoni* burrs. Further investigation is necessary before definite conclusions are reached with regard to the cause of this phenomenon. A number of possible experiments under natural and controlled conditions suggest

themselves in this connection. While the greenhouse environment was thought to be unsuitable for obtaining reliable genetical data, possibilities which might otherwise have been overlooked have been revealed as a result of it.

2. Cytology

It was thought that specific differences in *Xanthium* might be correlated with differences in chromosome number, as has been found to be the case in *Datura*, *Oenothera*, *Rosa*, and many other genera. Slides showing pollen mother cells in different stages of division have been studied in *X. italicum*, *X. pennsylvanicum*, and *X. inflexum* (the last probably heterozygous), as well as in two hybrids which were obtained by controlled pollination. As has already been explained, it has not been possible in the time thus far devoted to the investigation to prove, without doubt, the purity of the species under consideration. A preliminary survey of the chromosomes in the forms studied, however, would seem to indicate that the difference between species of *Xanthium* is probably a difference in factors, rather than a difference in chromosome number. The reduced number, in all the cases studied, was 18. Pl. XI *X* will suffice to show the size and general appearance of the chromosomes in the heterotypic metaphase. The five upper cells were from hybrid *X. inflexum* × *italicum;* the four below from *X. pennsylvanicum*. The drawings were made with the camera lucida and are shown in the photograph magnified 1220 diameters.

3. Physiology

As is well known, the two seeds in the normal *Xanthium* fruit differ in size. The smaller one is more protected by the inclosing burr. It was long ago observed that the larger, lower seed tended to germinate first. Rowlee (16), in 1893, mentioned the difference in the time of germination of the two seeds, and much has since been written upon the subject. As a result of the researches of Crocker, Shull, and Davis, reported at intervals since 1906, the factors responsible for delayed germination in *Xanthium* have largely been determined. An experiment in the McGill greenhouse during the winter of 1921 suggested that the determining differences between upper and lower seeds might not be equally great in all species of

Xanthium. This experiment involved about 150 burrs, which were sorted and planted in flats. Within about 10 days, one plant had been produced by most of the burrs. One type, however, consistently showed two seedlings from each fruit. It was therefore decided to make further observations with regard to this point, and general field notes upon delayed germination.

CROCKER (4) had pointed out that the burr was indirectly responsible for much of the delay in germination of the upper seed. Having shown that the coats of the upper seeds contribute to the delay by excluding more oxygen than those of the lower seeds, he says that the growth of the former in nature "comes about by a partial disintegration of the seed-coats. . . . . The length of the delay depends upon the ability of the seed-coat, protected by the surrounding burr, to resist the factors of disintegration in the soil. The portion of the burr covering the lower seed decays within a few months after burial, while the portion covering the upper seed is always far more persistent." More recently, MCHARGUE (9) has laid even greater stress upon "the mechanical conditions within the burr," arguing that these are sufficient to cause all the delay observed in his experiments.

In the summer of 1923 half the burrs were planted whole and half were opened. This gave equal numbers of whole burrs, lower and upper seeds, except where one seed was found to be lacking or was injured in the process of removal. The net result was as follows: Of 367 whole burrs planted 136 produced no plants that year (about 37 per cent), 220 produced one plant each (about 60 per cent), 11 produced two plants each (about 3 per cent). Of 278 lower seeds planted 221 produced plants (about 79.5 per cent), 57 produced no plants (about 20.5 per cent). Of 279 upper seeds planted 93 produced plants (about 33.3 per cent), 186 produced no plants (about 66.7 per cent).

SHULL (21) and others have pointed out that weeds generally have a very small percentage of non-viable seeds. The fact that 37 per cent of the whole burrs planted produced no plants in that season is partly to be accounted for by the prevalence of the *Xanthium* trypetid fly, especially in burrs from the south. When the burrs were opened for the removal of seeds, a very large proportion of them were

found to contain pupae of various ages. A number of these pupae were removed without injury and placed upon damp filter paper in a covered preparation dish. Two males and a female emerged and were identified by WILLEY as *Trypeta aequalis* Loew. This insect was discussed by MARLATT (8) in 1891 as a natural enemy of *Xanthium*, of wide distribution and of possible economic importance. He described the ecological relation between insect and plant, and observed: "The larva has never been known to occur in more than one

TABLE VII

No.	Source	Both good	Both spoiled	Lower good; upper spoiled	Upper good; lower spoiled	Upper			Lower		
						Good	With pupae	Shriveled	Good	With pupae	Shriveled
100..	Georgia	42	17	11	30	72	5	23	53	12	35
100..	Alabama	37	13	10	40	77	6	17	47	30	23
100..	Florida	28	12	6	54	82	10	8	34	39	27
50..	South Carolina	29	2	5	14	43	1	6	34	8	8
50..	North Carolina	37	1	6	6	43	1	6	43	5	2
325..	Ohio	256	6	27	36	292	4	29	283	11	31
50..	Montana	25	7	8	10	35	7	8	33	11	6
100..	Kansas	44	7	20	29	73	6	21	64	13	23
50..	Illinois	40	3	2	5	45	0	5	42	1	7
75..	Wyoming	33	16	12	14	47	12	16	45	11	19
1000		571	84	107	238	809	52	139	678	141	181
Percentage		57.1	8.4	10.7	23.8	80.9	5.2	13.9	67.8	14.1	18.1
Probably		57.1	8.4	10.7	23.8	80.9	19.1		67.8	32.2	

of the two seeds normally contained in the *Xanthium* burr." Abundant material being on hand, it was thought that it would be of interest to determine (1) to what extent the burrs had been attacked by this insect, (2) whether the insect ever destroyed both seeds, (3) whether the insect showed any preference for the larger lower seed. A thousand burrs from the same stock used for planting, which included six different types from ten different states, were opened and examined with a view to deciding these points. The results are summarized in table VII.

When both seeds were spoiled, it was probably in most cases due to two insect punctures, one penetrating each ovule. Several times two well developed pupae were found in one burr, each entirely fill-

ing one seed cavity. Usually only one puncture was made in a burr. The lower seed was more often destroyed in this way than the upper, but there was no evidence that the insect follows a regular course of action in this respect. The fact that more than 32 per cent of the lower seeds were here found to be spoiled, helps to account for the low percentage of germination from whole burrs, already noted. The fact that one-fifth of the lower and two-thirds of the upper seeds planted failed to produce plants is still to be explained. Many of these failures were in the southern forms (*X. chinense* Mill. 10). These have small seeds, and it is probable that they were planted too deeply. Later, when this was suspected, part of the earth was removed in some of the rows and more seeds then germinated. The fact that under the same conditions a much larger percentage of lower seeds germinated supports the findings of CROCKER and SHULL (18, 19, 20). One-third, however, of the 279 upper seeds planted did germinate without delay.

SHULL and DAVIS (21) discuss possible reasons for the fact that MCHARGUE did not find the normal percentage of delay in the first year. Of the four suggested possibilities, the only one which might apply to this case is that the coats may have been injured by handling. Care was taken not to break the seed or cut the seed coat. Many seeds were prepared for planting at one time and wrapped in soft paper, however, the ends of each small package being labeled. When opened and planted, the coats were apparently intact; 33 per cent of the upper seeds thus planted germinated within about 10 days, while in the same period only 3 per cent of the whole burrs planted produced two seedlings each. This difference could hardly all be accounted for by the factors noted. It seems clear from this, and from the next year's results, that the burr factor is an important one in causing delay in nature.

In 1924 most of the burrs were planted whole; in one part of the garden seeds from opened burrs were sown. The records of planting were not kept in the same way as in 1923, but the general result was noted. There was a marked difference in the germination percentage of burrs and seeds gathered in the fall and stored in the laboratory until planting time, and that of those gathered on the shore in the spring. Of the latter, 213 burrs were planted, 67 of them producing

two plants each. Of the former, 442 burrs and seeds were planted; 45 burrs produced two plants each, while 26 upper seeds from opened fruits also germinated. More than 30 per cent of the burrs which had been exposed to outside conditions during the winter produced two plants each. Of the ones which had been stored dry, most of those which produced two seedlings were *X. pennsylvanicum*. In one case, from a single plant of this species, 11 burrs were sown and 8 of them produced two equally strong plants.

McHargue says, "Plants from large seeds are in every respect larger and more vigorous; this difference maintains after 60 days."

TABLE VIII

	Plants equal	One plant slightly larger; both strong	One plant distinctly stronger
Separated.........	69	34	11
Unseparated.......	13	7	1
Total.........	82	41	12

Under the conditions in the garden this was not generally the case. Two hundred and seventy paired plants, from 135 fruits, some of them from the miscellaneous burrs washed ashore and gathered in the spring, and others from burrs from known mother plants, were kept under observation during the summer. In the fall they were compared as to size, productiveness, and other characters. Twenty-one of these had been left unseparated throughout the season. All 270 plants were strong, with branches in every leaf axil and abundance of fruit. Numerical results are given in table VIII.

While the duplex seedlings occurred most frequently in *X. pennsylvanicum*, they were produced occasionally by other species. They were seldom observed in *X. italicum*. Generally, however, the tendency was seen in a number of burrs from one parent, rather than in isolated cases. It seemed to be a varietal difference.

Bitter grew *X. italicum* under observation and separated three races by their stem color, with which he found correlated other physiological differences; notably the time of flowering varied directly with the amount of color in the stem. Differences in stem color were

also noted by the writer among plants of *X. italicum* from different parents. The general habit, especially the angle of branching, also varied.

Another varietal difference which has already been mentioned, was that of the leaf color in types *A* and *B* of *X. curvescens*. Leaves of each kind were gathered and examined in section under the microscope. No very great difference in structure being noted, it was decided to extract and compare the pigments. Leaves were dried and powdered, and equal weights of the two kinds were taken. The directions of ONSLOW (15) were followed in the extraction and separation of the pigments. The latter were then compared with a colorimeter, at least 20 readings being taken and averaged in each case. It was found that type *B* leaves contained more of chlorophyll α, carotin, and xanthophyll, while there was slightly more of chlorophyll β in the lighter green leaves of type *A*. The ratios were as follows:

	A	B
Chlorophyll α	75	100
Chlorophyll β	100	96
Carotin	65	100
Xanthophyll	85	100

As the growing plants have been observed during the past two years, many new problems have presented themselves. Some of them have been indicated in this paper. The writer hopes to continue the investigation and to obtain further results along these lines.

Summary

1. Species of *Xanthium* found commonly on the shores of the St. Lawrence River at Montreal are *X. italicum* Mor., *X. pennsylvanicum* Wallr., *X. inflexum* Mack. & Bush, and a form thought to be *X. orientale* Linn., but of the described American species most like *X. curvescens* Millsp. & Sherff.

2. These species all blossom at about the same time, and experiment has shown that hybridization is possible between them.

3. Plants of *X. italicum* with some burrs of the *X. Wootoni* type have been found wild here. They fit the description of the "duplex specimen" found by COCKERELL in New Mexico.

4. Plants of all the species studied and some hybrid forms, when forced in the greenhouse, produced some *X. Wootoni* burrs.

5. In hybrids, the characters of *X. italicum* apparently are dominant to those of any of the other three species grown here.

6. In the species and hybrids thus far investigated, the reduced chromosome number was found to be 18.

7. In garden observations upon delayed germination during two summers, it has been found that the burr is an important factor in causing delay in nature.

8. MARLATT'S suggestion that *Trypeta aequalis* Loew is an important natural enemy of *Xanthium* has been confirmed by observations upon 1000 burrs from the southern United States. It was found that sometimes both seeds in a burr were attacked by the insect, although it was usually only one, and more often the lower.

9. In the garden, when both seeds in a single burr germinated, the two seedlings were often equal in size and strength, while only rarely was one much more vigorous than the other.

10. The tendency to produce two plants the first season seemed to be characteristic of some varieties. Other varietal differences were noted among progeny of different plants of the same species.

The writer wishes to express thanks to Professor F. E. LLOYD for his constant interest, and for his aid in photographing the specimens; to thank Professor C. M. DERICK for valued suggestions and encouragement, Dr. J. N. ROSE under whose personal direction the facilities of the National Herbarium at Washington were enjoyed, the Directors of the Agricultural Experiment Stations in the United States and Canada, and all who cooperated in the collection of material.

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DESCRIPTION OF PLATES IX–XI

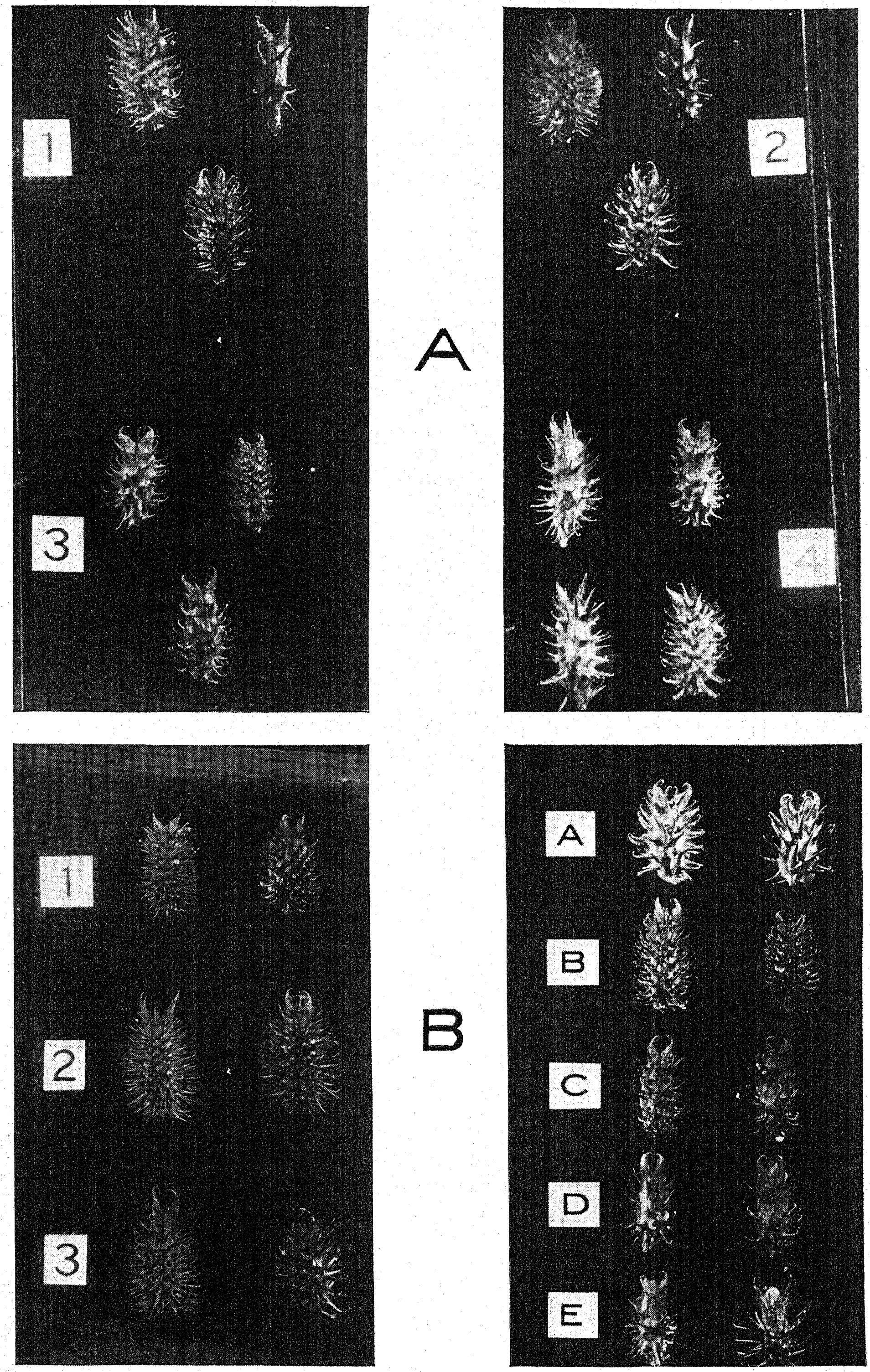
PLATE IX

A: 1, 2, 3, 4.—Above, fruits of different character found on same plant; below, typical fruit or fruits produced in next generation by seeds from burrs of both kinds.

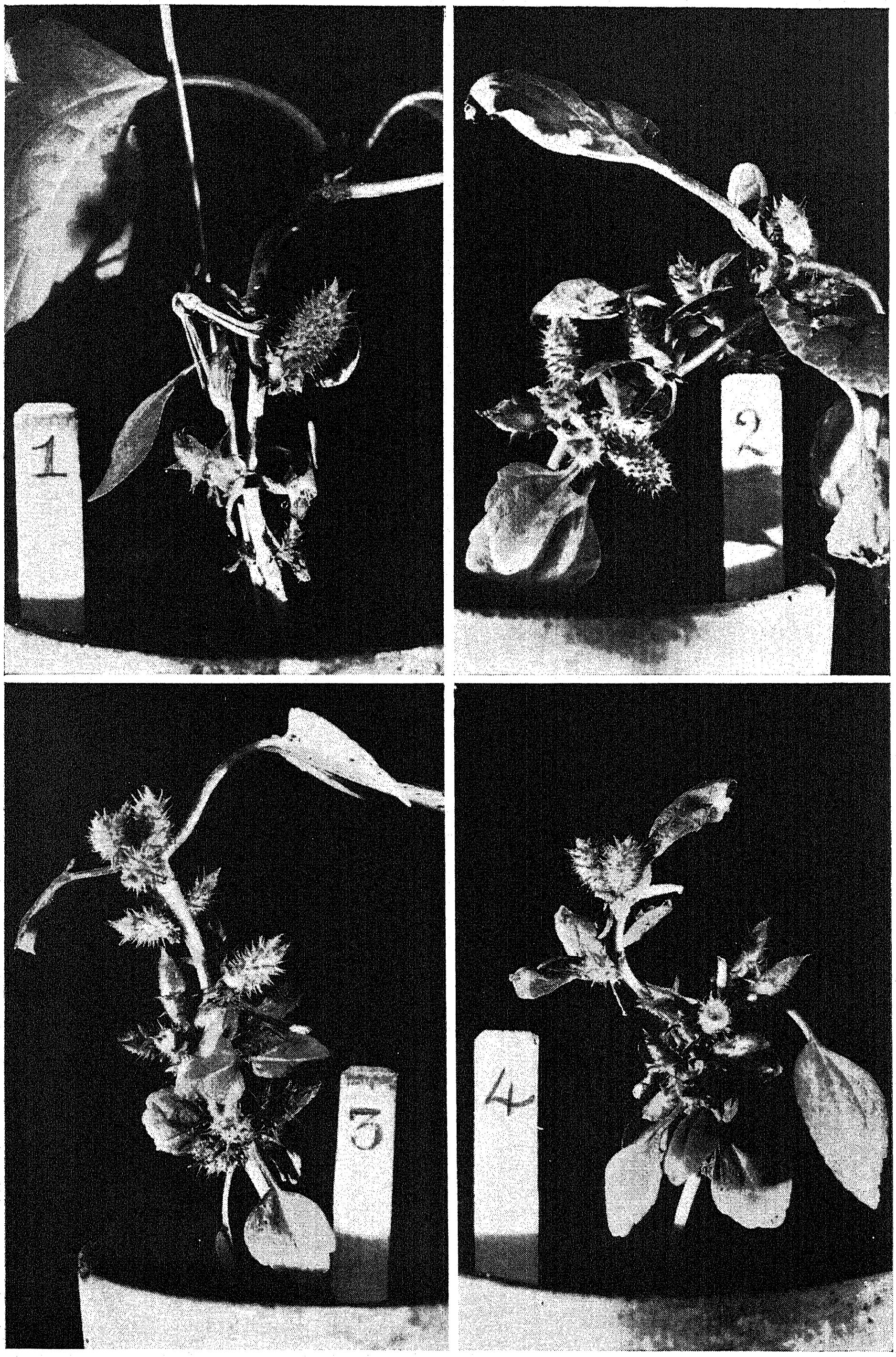
B.—1, *X. italicum* Mor.; 2, *X. pennsylvanicum* Wallr.; 3, *X. inflexum* Mack. & Bush; types a, b, c, d, e, *X. curvescens* Millsp. & Sherff.

PLATE X

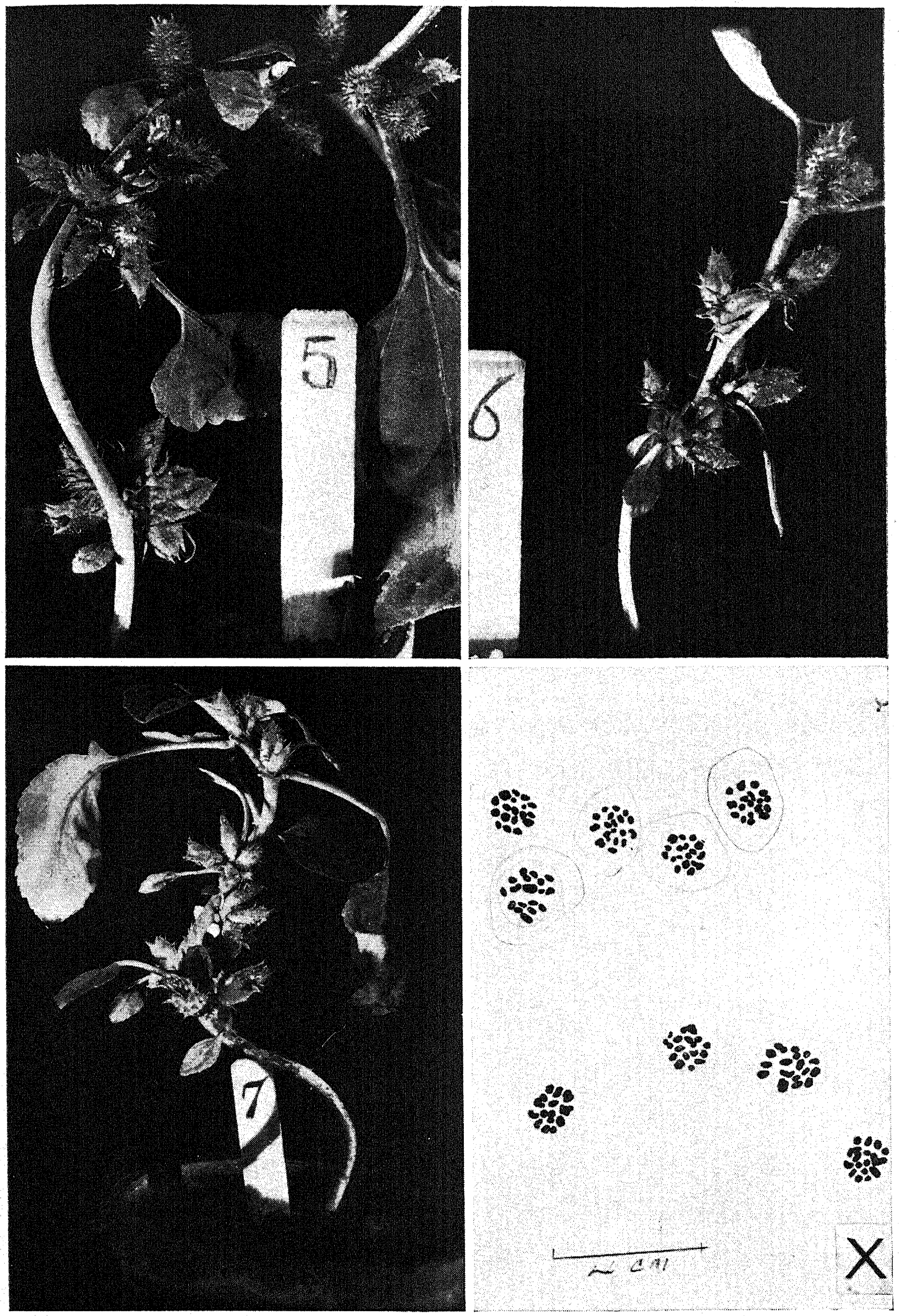
FIG. 1.—Hybrid obtained by crossing *X. italicum* and *X. inflexum:* only two fruits were left to develop; upper one a typical *X. italicum* burr, lower one quite abnormal.



SYMONS on XANTHIUM



SYMONS on XANTHIUM



SYMONS on XANTHIUM

FIG. 2.—Hybrid obtained by crossing a variable form of *X. italicum* and *X. pennsylvanicum:* normal fruits of *X. italicum* type.

FIG. 3.—Hybrid obtained by crossing *X. italicum* and *X. inflexum:* normal burrs have *X. italicum* characters (parent plants different from those used in fig. 1).

FIG. 4.—Hybrid obtained by crossing *X. italicum* and *X. curvescens B*: lower clusters show burrs resembling *X. Wootoni*, while rather small *X. italicum* fruits are seen at top of plant.

PLATE XI

FIGS. 5, 6, 7.—Plants of pure species, selfed, which produced burrs of *X. Wootoni* type when grown in greenhouse; fig. X, chromosomes in heterotypic metaphase; magnification 1220 diameters; above, five pollen mother cells from hybrid *X. inflexum* × *italicum;* below, four pollen mother cells from *X. pennsylvanicum.*

POLLEN DEVELOPMENT IN THE APPLE, WITH SPECIAL REFERENCE TO CHROMOSOME BEHAVIOR[1]

J. S. SHOEMAKER

(WITH PLATES XII–XIV)

Introduction

Fruit development in the apple is dependent in large degree upon successful pollen development and subsequent fertilization. The quality of pollen available for fertilization may affect not only the fruitfulness of existing varieties, but also may influence the germinal constitution of the progeny. Investigators, however, have been concerned more with pointing out abnormalities affecting fruitfulness in the apple than with attempting to determine underlying causes.

A study of male gametogenesis of certain varieties in which a high percentage of normal pollen is found, and of others where indications of abnormal pollen development are outstanding, should show the time and sequence of manifested irregularities, and furnish a basis of interpretation of the possible causes. The varieties of the Winesap group (Winesap and its seedlings) are noted for self, inter, and cross sterility, and are characterized further by a low percentage of pollen germination. On the other hand, Delicious pollen is of high germination capacity and is very successful in cross fertilization. The purpose of this study is to investigate chromosome behavior with reference to pollen development in Delicious and in certain members of the Winesap group.

The status of the problem is indicated by a review of previous investigations on the relation of size of pollen grain to viability, the comparative value of Delicious and Stayman Winesap pollen in controlled pollination studies, and chromosome number and behavior in Rosaceae.

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Relation of size of pollen grain to viability

Germination studies of apple pollen were conducted by BEAUMONT and KNIGHT (5), who found that pollen grains which did not germinate in artificial media could be divided into the following four classes:

1. Shrunken and shriveled grains without apparent cytoplasm.

2. Small round grains with cytoplasm, but having a diameter of only one-half to three-fourths the diameter of normal pollen grains. These swell slightly and occasionally burst.

3. A small class of grains which average much larger than the normal grains and are filled with cytoplasm. These grains do not ordinarily produce tubes, or if they do so the tubes are abortive.

4. Grains normal in size and apparently in contents, which produce tubes that burst after attaining a length of approximately two pollen grain diameters.

TABLE I

AVERAGE PERCENTAGES OF POLLEN GERMINATION OF THREE MEMBERS OF WINESAP GROUP AND DELICIOUS

Variety	Total number observations	Average percentages pollen germination
Paragon*	29	28.3
Stayman Winesap	37	36.9
Winesap	17	65.5
Delicious	38	88.5

*This variety was purchased from a nurseryman as Gilbert Winesap, and was mentioned by BEAUMONT and KNIGHT as such, but has later been identified as Paragon.

The average percentages of pollen germination of various apple varieties also were studied by BEAUMONT and KNIGHT, and considerable variation was found. The average percentages of germination denoted differences not only between individual varieties, but also between the members of the Winesap group and Delicious. Table I, adapted from BEAUMONT and KNIGHT, indicates the average percentages of pollen germination of Paragon, Stayman Winesap, Winesap, and Delicious.

The studies and data of BEAUMONT and KNIGHT indicate three salient features pertinent to the present investigation: (1) that apple pollen grains can be divided into distinct classes according to size; (2) that there is some correlation between size and viability of pollen

grains; and (3) that the percentage of pollen germination of the members of the Winesap group is considerably lower than that of Delicious.

Comparative value of Delicious and Stayman Winesap pollen in controlled pollination studies

The low and high average germination percentages previously mentioned for Stayman Winesap and Delicious suggest that certain internal factors may have influenced the results. This point is

TABLE II

PERCENTAGE SET OF FRUIT WITH BOTH DELICIOUS AND STAYMAN WINESAP USED AS POLLEN PARENTS IN CONTROLLED CROSSES

POLLEN VARIETY	STATION	PERCENTAGE SET OF FRUIT
Delicious	Minnesota	9.28
	Washington	7.09
	Ohio	20.01
		Average 12.13
Stayman Winesap	Minnesota	3.34
	Washington	0.00
	Ohio	6.60
		Average 6.31

emphasized by a consideration of the comparative value of the two varieties as pollen parents in pollination work. Investigations conducted by POWELL (31) indicated that Stayman Winesap and Paragon were both self and inter sterile. Further tests by CLOSE (9), BALLARD (4), GOWEN (19), DORSEY (14), MORRIS (29), AUCHTER (2, 3), KEIL (21), and WILLIAMS[2] (43) showed that the members of the Winesap group were self and inter sterile. Pollination data from various stations, where both Delicious and Stayman Winesap were used as male parents in controlled crosses, have been summarized from reports of DORSEY, MORRIS, KIEL, and WILLIAMS, and are given in table II.

At these stations the pollen of Delicious was used on 19 varieties and that of Stayman Winesap on 17. It is evident from the data

[2] Director's Annual Report.

presented in table II that in a relatively great number of pollinations on many varieties, and under approximately similar conditions, Delicious as a pollen parent was considerably more successful than Stayman Winesap.

Chromosome number and behavior in Rosaceae

Workers have investigated chromosome number and behavior in Rosaceae rather extensively, and have reported numbers for a few economic fruits of the family. In the strawberry, VALLEAU (40) found the haploid number to be 26. In the plum, DORSEY (12) reported 10 haploid chromosomes for a number of species and species hybrids. KNOWLTON (24) stated that in the J. H. Hale peach, a self sterile variety in which a high percentage of aborted pollen occurred, the haploid chromosome number was 8. Investigations in *Rubus* by LONGLEY (25) and LONGLEY and DARROW (27) showed that the basic number was 7 for this genus. In *Potentilla rupestris* and *P. sylvestris* haploid numbers of 8 and 16, respectively, were found by FORENBACHER (in WILSON 44). MURBECK (in WILSON 44) believed that the haploid number in *Alchemilla arvensis* and *A. grossidens* was 16. In *Crataegus monogyna*, MEYER (28) found the haploid chromosome number in the pollen mother cells to be 16. In a study of many species of *Crataegus*, LONGLEY (26) observed gametophytic chromosome numbers of 16, 24, and 32. In *Rosa*, TÄCKHOLM (38, 39), BLACKBURN and HARRISON (7), and PENLAND (30) found 7 as the fundamental haploid chromosome number for the genus.

From a survey of the preceding genera of Rosaceae, it is apparent that they may be grouped into three classes with respect to chromosome numbers: (*a*) with 8, or multiples of it, as the haploid number (peach, *Potentilla* spp., *Alchemilla* spp., and *Crataegus* spp.); (*b*) with 7 as the basic number (*Rosa* and *Rubus*); and (*c*) with neither 7 nor 8 as the basic number (plum and strawberry).

In triploid, pentaploid, and hexaploid forms of *Rosa*, BLACKBURN and HARRISON observed that in many cases the split univalent chromosomes failed to reach the poles, and formed micronuclei. They believed that hybridity in *Rosa* was evident from the irregular chromosome distribution and resultant abnormal pollen

grains. PENLAND found in the diakinesis stage of *Rosa*, that besides a certain number of bivalent chromosomes, many univalents were present also. He thought that this might be due to the addition of parental chromosomes, or to the partial conjugation of unequal sets of chromosomes. According to him the probable explanation of these conditions was incompatibility, either quantitative or qualitative. PENLAND furthermore showed that as early as the anaphase it was apparent that the unpaired chromosomes had either lost their motility, or else were not subject to the same forces as the bivalents, for they were found free from it and out in the cytoplasm. Sooner or later, however, the majority of them became massed about the equatorial plate, following the bivalents (somewhat later) into the telophase, thus giving an anaphase two distinct steps. As a result of this lagging, many of the univalent chromosomes were left outside the membrane of the daughter nuclei. In subsequent divisions of the pollen mother cell they were found to produce separate spindles of their own. TÄCKHOLM (39) concluded from studies of *Rosa* that the chromosome conditions found in the polyploid forms indicated hybrid origin. He believed that pollen sterility in *Rosa* was the outcome of latent hybridity, and that pollen grains which received bivalent and a few univalent chromosomes were viable.

LONGLEY (26) found that the species of *Crataegus* could be divided into three major classes : (1) diploid species characterized by normal pollen formation; (2) triploid and tetraploid species that were able to develop their pollen mother cells to the tetrad stage (these showed irregularities in their chromosome distribution that frequently led to polyspory and polycary); and (3) triploid and tetraploid species in which the pollen mother cells had thin and vacuolated cytoplasm and seldom developed to the tetrad stage (these represented hybrids that had resulted from crosses of distantly related species). The characteristics shown by these polyploid forms, namely, pollen sterility, irregular chromosome distribution, polyspory, and polycary, were considered by LONGLEY to be features associated with hybrid forms.

A study by LONGLEY (25) of pollen mother cells of various species and forms of *Rubus* indicated that they could be divided into

two major classes, according to chromosome number and behavior: (1) diploid, in which the gametophytic number was 7; and (2) polyploid, including triploid, tetraploid, pentaploid, hexaploid, and octaploid, in which haploid chromosome numbers of 14 and 21/2 occurred. Many of the New England species of *Rubus* were found by him to belong to the triploid group. The diploid class was characterized by normal distribution of chromosomes and pollen formation, and the polyploid class by striking irregularities in chromosome behavior and pollen development. In a later paper, LONGLEY and DARROW (27) discussed the irregular distribution of chromosomes in the polyploid raspberry variety La France. During the reduction division some of the chromosomes lagged on the spindle and were extruded into the cytoplasm, and remained outside the daughter nuclei. The resultant pollen grains varied in chromosome numbers and in viability, which they believed to be indicative of hybrid origin. The same investigators reported a second group of sterile raspberries including all but two of the polyploidous forms. At least six forms were sterile and were characterized by an unequal distribution of the chromosomes in tetrad formation. They believed that sterility in these forms might be attributed to an unbalanced chromosome condition in the nuclei of the pollen grain.

The study herein reported was made to determine the chromosome number of certain apple varieties, to trace the chromosomes through the various stages of normal gametogenesis, and to investigate abnormalities which occur in pollen development.

Methods

Three varieties of apples were selected for cytological study of pollen development, Stayman Winesap, Paragon, and Delicious. Twigs of these were taken from the orchard of the Minnesota State Fruit Breeding Farm, Zumbra Heights, and forced in a greenhouse at University Farm, St. Paul. Additional material was collected directly in the orchard. Buds of each were killed at intervals from the time of the first swelling until the individual flower buds of the cluster were fully formed. Each bud was separated from the cluster and the epidermal hairs removed in order to facilitate fixation and subsequent cutting. ALLEN'S (1) modification of Bouin's solution

was used as the killing and fixing fluid. This gave good results in nearly all stages. In the early spireme and synapsis stages, however, there seemed to be a tendency for the chromatin to become disorganized with this fixative. The material was prepared for study by the paraffin method, stained with Haidenhain's iron-haematoxylin, and cut with the microtome at thicknesses within the range of 5–12 μ.

Pollen grain measurements of the three varieties were made at the Fruit Breeding Farm. Anthers with long filaments were snipped off and the pollen teased out in drops of lactic acid on 25×75 mm. glass slides. The lactic acid was employed to afford a liquid medium which would prevent bursting and germination of the pollen grains. The pollen grains of each variety were outlined with the aid of the camera lucida, measured in millimeters, and later calculated to their proper size in microns. The greatest diameter, usually from a germ pore to the opposite side of the triangular pollen grain, was considered as the basis of measurement. In grains which were more round than triangular this method could generally be followed. One thousand pollen grains of each variety were measured and grouped into classes according to diameter.

Normal pollen development

Pollen mother cells

In a given loculus of an anther the pollen mother cells of the apple show little variation in stage of development, although some are slightly more advanced than others. Different loculi of the same anther, however, frequently attain different stages of development, and the variation between loculi of other anthers of the same flower is still greater. Thus within a section of a flower stages ranging from resting pollen mother cells to tetrads may be observed. Within the cluster of buds a greater range is found, the central leader being considerably more advanced than the laterals. GATES (16) believed that irregularity in stages of development of cells in the loculi probably was connected with the failure in pollen development in forms of *Oenothera*.

In material killed April 24, 1924, the sporogenous cells of the anthers were in the rounded resting pollen mother cell stage (fig. 1), and had evidently passed the winter in this stage or just previous

to it. The pollen mother cells are easily distinguishable from the adjoining tissues by their central position, size, form, and staining reaction. The nucleus is large and the cytoplasm dense and granular. The nucleolus is probably the most conspicuous feature of the nucleus, and appears as a roundish, darkly staining sphere. The chromatin network of the resting nucleus is delicate and finely granular.

In Delicious anthers killed at a later date, the pollen mother cells and the tapetum are distinctly separated (fig. 2). There has been an apparent although perhaps not actual increase in the number of pollen mother cells, and they now number approximately 25 in cross-section. The size of the individual cells, nuclei, and prominent nucleoli also appears larger. The reticulum of the nucleus in these cells is now more granular and distinct, due to the aggregation of the chromatin into more dense threads.

When the pollen mother cells enter synapsis, the chromatin concentrates into an irregular densely staining mass at one side of the nucleus (fig. 3). Occasional threads may be seen protruding from the aggregated mass. Following synapsis the double spireme is formed and the diakinesis stage is initiated.

Heterotypic and homotypic divisions

During the prophase the spireme shortens, thickens, and separates into paired chromosomes, which during diakinesis are distributed around the periphery of the nucleus. Counts of chromosomes in numerous cells at this stage indicate that the haploid number in Delicious is 14. While the apple does not lend itself readily to a study of the double nature of the chromosomes, certain manners of pairing were observed (fig. 11). The paired chromosomes in diakinesis of Delicious are shown in fig. 8, and of Stayman Winesap in fig. 9. In Delicious the spireme seems to shorten and thicken into a definite number of paired chromosomes, which may readily be counted. In Stayman Winesap, however, the chromosomes appear more irregular and variable in size and form. Usually one nucleolus is present in the nucleus during the diakinesis period. It is relatively large, and is generally found toward one end of the somewhat oval nucleus. Occasionally two nucleoli may be seen in the nucleus. When this occurs they appear smaller than when only one is present. The

disappearance of the nucleolus, the dissolution of the nuclear membrane, and the formation of the bipolar spindle terminate the diakinesis stage.

The period between diakinesis and the metaphase of heterotypic division is of extremely short duration. At metaphase the small, somewhat oval chromosomes arrange themselves on the equatorial plate. For a time they lie very close together, but also become separated, and then the pairs may readily be counted. The chromosomes of Delicious always seem to behave regularly, and it is relatively easy to trace them through the various division stages. At metaphase and anaphase the chromosomes lie in slightly different planes. Fourteen haploid chromosomes were counted for Delicious in these stages (figs. 12–15), and checked against the counts made in diakinesis. The fibers of the bipolar spindle extending to the poles are distinct, and terminate in points in the cytoplasm short distances from the plasma membrane. They become less thick in appearance, however, as the close of heterotypic division approaches. Soon after the chromosomes reach the poles, the daughter or dyad nuclei are organized, and preparation is made for the second division.

The fibers of the homotypic division spindles are far less distinct than those of the heterotypic division. After reorganization the dyad nuclei usually divide simultaneously to form the tetrad nuclei. Walls are laid down and the cells spread apart, thus separating the four microspores and allowing the entrance between them of clear, viscid sap.

Pollen grains

At first the microspores are inclosed within the tetrad wall. A period of growth characterized by rounding up of the microspores then occurs. Subsequently the tetrad wall breaks down and the microspores are set free in the loculi of the anther. A short time before the anther is ready to dehisce, the microspore nucleus divides to form the generative and vegetative nuclei of the pollen grain. During and subsequent to this division, the walls of the microspores become greatly thickened and corrugated, until finally the structure of the mature pollen grain results.

Abnormal pollen development

Irregular chromosome behavior in maturation

The regular heterotypic and homotypic divisions of the pollen mother cells previously described are exemplified in Delicious. In Stayman Winesap, on the other hand, irregular chromosome shape, number, and distribution occur. Such irregular chromosome behavior is similar in many respects to that outlined previously in references to the studies in various forms of Rosaceae by Blackburn and Harrison (7), Täckholm (39), Penland (30), Longley (25, 26), and Longley and Darrow (27).

Studies in *Oenothera* are of interest in connection with the occurrence of lagging chromosomes in Stayman Winesap. Rosenberg (32, 33) showed that in hybrids of *Drosera longifolia obovata* with *D. rotundifolia* and *D. longifolia*, some of the smaller chromosomes were left behind in the cytoplasm in the first and second divisions, where they formed small nuclei. In a later work Rosenberg (34) studied a cross of *D. longifolia*, having 40 diploid chromosomes, with *D. rotundifolia*, having 20 diploid chromosomes. At reduction division in the pollen mother cells of this hybrid, 10 bivalent chromosomes and 10 univalents were observed. The bivalents divided normally and traveled to the poles. The univalents, however, passed at random to the poles or remained in the cytoplasm. When they were left in the cytoplasm they proceeded to form other nuclei. The nuclei thus formed were smaller than the ones formed in the usual manner from heterotypic division. In *Oenothera* hybrids Gates (16) observed bodies in the cytoplasm, and concluded that they were chromosomes which had become separated from their fellows on their way to the poles of the spindle. He also found chromosomes in the cytoplasm outside the nuclear wall of tetrad cells, which might or might not be surrounded by a "nuclear membrane." In 21-chromosome offspring of *Oenothera lata* × *O. gigas*, *O. Lamarckiana* × *O. gigas*, and *O. gigas* × *O. Lamarckiana*, Geerts (18) observed the occurrence of 7 pairs of bivalents and 7 univalent chromosomes. In the first division of the former the usual separation of the two members of each of the 7 pairs and their passage to the poles occurred. Geerts found that in the 7 unpaired chromosomes the second

division might be irregular, and some or all of these might fail to reach the pole, and hence would remain outside the daughter nuclei. He further observed that a number of irregular chromosomes or pieces of chromosomes might be distributed to each of the four homotypic poles. In regard to the fate of these fragments of irregular bodies, he stated that when the tetrad nuclei formed, this chromatin material was occasionally taken up by the nucleus, although it usually remained outside the nuclear membrane. Furthermore, he claimed that this chromatin material which remained outside the nucleus often developed into small nuclei in the young pollen grains. In the usual 7 and 8, and in the rare 9 and 6 forms of *O. lata* and *O. semilata*, GATES and THOMAS (17) found that the extra chromosomes were frequently left behind, and could be seen fragmenting and degenerating in the cytoplasm.

In *Triticum polonicum* × *T. spelta* hybrids, KIHARA (22) noted 14 bivalent and 7 univalent chromosomes in the heterotypic prophase, and that the latter were distributed irregularly at the second mitosis. KIHARA (23) further observed lagging univalent chromosomes at heterotypic division in triploid wheat hybrids, which either passed at random to the poles, or remained behind in the cytoplasm and took no further part in nuclear divisions. In cytological studies of wheat crosses, WATKINS (41) also noted lagging chromosomes which first appeared during heterotypic division. They were usually less than three in number. During the homotypic division the univalent chromosomes were found to pass to the poles at random, and very often one or more of them failed to reach the poles and were left outside the daughter nuclei in the cytoplasm. SAX (36) thought that sterility in *Triticum* hybrids could be explained on the basis of (1) the proportion of univalents to bivalents in the resulting gametes, and (2) the completeness of chromosome sets as determined by both the univalents and bivalents. The former factor accounted for sterility in the F_1, where it was assumed that only those gametes are functional which approach in chromosome numbers the gametes of the parents. The latter factor influenced the F_2 generation, as complete sets were not always found to be present. In the F_2 individuals weak somatic development partially prevented gamete formation, thus adding its effect to sterility due to chromosome combinations.

Heterotypic division

In Stayman Winesap in the diakinesis stage certain deviations from the normal as exemplified in Delicious may be observed. Although good spireme preparations were not plentiful in which to observe any irregularities at this stage, certain features of diakinesis indicate that perhaps not all of the abnormalities of the spireme were due to poor fixation. In diakinesis the chromosomes are arranged around the periphery of the nucleus, although they are irregular in size, shape, and distribution. Some of them appear abnormally large, as though the spireme has failed to fragment completely, and two or more of the bivalents may have remained joined. Others are much smaller than the average and are probably univalents. The univalents often exhibit odd shapes similar to the figures assumed by the chromosomes in late metaphase. In a number of instances the chromosomes, as in fig. 10, appear to be outside the nuclear membrane before the latter structure has completely disappeared. The chromosomes of Stayman Winesap were difficult to count, but in a number of instances, when they were rather evenly distributed throughout the nucleus, counts indicated that at least not all of them were paired and that there were a number of univalents.

During the anaphase and telophase stages of the heterotypic division, the chromosome distribution is extremely irregular in Stayman Winesap. Many of the chromosomes lag on the spindle, and are left outside the daughter nuclei when these organize. Occasionally only one well defined nucleus is formed, and the chromosomes allotted to the other are distributed almost at random throughout the cytoplasm. The lagging chromosomes often organize as miniature nuclei and may persist for some time. Some of the chromosomes lost in the heterotypic division seem to be reincluded in the homotypic division.

Homotypic division

The homotypic division in Stayman Winesap shows considerable irregularity in chromosome behavior. During the interkinesis period fragments of one or more chromosomes, or entire univalents or bivalents, may be observed in the cytoplasm. Figs. 16, 17, and 18 are typical of interkinesis in Stayman Winesap. In fig. 16 the two nuclei are formed, but a large nucleolus is still present in the cytoplasm.

While it may be noted that GATES (16) found nucleoli which took only the orange of the iron-haematoxylin-orange stain, the large one present in this cell stains the intense color typical of chromatin. There is no evidence in the apple, however, that nucleoli are associated with the occurrence of chromosomes in the cytoplasm. In certain cases (figs. 16–18) many chromosomes appear in the cytoplasm during interkinesis. Some are organized as nuclei and others have not progressed so far. Examination of such cells shows that the number of chromosomes in Stayman Winesap at this period exceeds the number for Delicious. It has been pointed out that the chromosomes in Stayman Winesap are not all paired; some are univalents and others bivalents. While counts seemed to indicate more than 28 chromosomes in the dyad of Stayman Winesap, the material at hand was not sufficient to warrant a detailed study of the numbers of bivalents and univalents.

Coarsely knotted, loosely arranged chromosomes resembling spiremes are conspicuous and characteristic of Stayman Winesap (fig. 19). During this time fragmented pieces of chromatin organized into small nuclei appear in the cytoplasm (figs. 20, 25). In the early metaphase stage strands of chromatin may be seen extending from the equatorial plate (figs. 21, 24). During anaphase and telophase chromosomes are found lagging on the spindle, and many of them are left in the cytoplasm when the tetrad nuclei form (figs. 22, 23).

ABNORMALITIES FOLLOWING MATURATION

The extrusion of chromosomes into the cytoplasm during division precedes tetrad and microspore abnormalities. Such abnormalities play a significant rôle in pollen development in Stayman Winesap. Peculiarities in this variety, as contrasted with Delicious, are polyspory or the formation of extra microspores within the tetrad wall, polycary or the presence of additional nuclei in the microspores, and certain types of pollen grain degeneration.

POLYSPORY

Abnormal microspore number within the tetrad wall is known in various plants. A rather extensive list of plants with abnormal microspore number is given by COULTER and CHAMBERLAIN (10).

Among the forms investigated by WILLE (42), *Prunus cerasus* is mentioned as containing 5 microspores. Cases in which there were less than the usual number of 4 microspores, namely 2 or 3, were also found by WILLE. BEER (6) showed that in *Fuchsia* 6, 8, and even 10 microspores might result from the same pollen mother cell, due to irregular chromosome distribution during the anaphase. In *Rosa*, PENLAND (30) observed as high as 10–12 microspores which came from the same pollen mother cell. KIHARA (23) found in *Triticum vulgare-secale* hybrids that 2–6 microspores were sometimes formed from a single pollen mother cell.

Explanations of irregular microspore number in *Hemerocallis fulva* were offered by STRASBURGER (38), JUEL (20), and FULLMER (15). The failure of chromosomes to pass to the poles at the first mitosis was believed by STRASBURGER to give rise to small microspores. JUEL agreed with STRASBURGER, and also found that single chromosomes which had become separated from the rest might divide and give rise to nuclei and organize cells. FULLMER attributed the supernumerary microspores in *Hemerocallis fulva* to the division of one or more members of the tetrad. GATES (16), however, thought that extra mitosis would not account for such conditions.

More than the usual number of 4 microspores sometimes occur in Stayman Winesap (figs. 32–34). Six microspores have been observed within the tetrad wall. These supernumerary microspores appear normal except in size. Abnormalities of this nature have never been observed in Delicious. While it is possible that one or more of the 4 microspores divide and give rise to additional ones, as found by FULLMER, no evidence of this was observed in the apple. It is more likely that the abnormal numbers observed in Stayman Winesap are a result of irregular meiotic divisions.

It is probable that the chromosomes which appear in the cytoplasm during homotypic division become organized into nuclei and give rise to extra microspores. These upon liberation from the tetrad wall may appear as small pollen grains. Evidence will be presented in a later section to show that small pollen grains are found, and that they occur more frequently in Stayman Winesap than in Delicious.

Polycary

Additional nuclei were frequently observed in the microspores of Stayman Winesap, giving rise to the condition known as polycary. Such abnormalities are undoubtedly due to irregular division, as they have never been noticed in Delicious, where regular division is the rule.

Dorsey (12) found in the plum that following heterotypic division some abnormalities appeared in nuclear reorganization. These variations included the formation of as many as three nuclei in the place of one, or rarely the organization of one large and one small nucleus in a single microspore. Sometimes an unusually large nucleus was formed somewhat in advance of the others. Following the liberation from the tetrad wall, the unusually small microspores sometimes found appeared to him to complete the series of variations from the condition noticed in most of the other forms. Penland (30) found in *Rosa* that the so-called univalent chromosomes with a separate nuclear membrane might be caught within the wall of a larger microspore.

The two figures to be considered illustrate polycary in Stayman Winesap. In addition to the usual relatively large nucleus in each microspore of fig. 30, there appears in two cases an additional small nucleus, and in the third microspore two smaller nuclei are visible. One of the microspores of fig. 31 shows three miniature chromatin bodies in a small tetrad microspore. Such extra nuclei have never been observed in the mature pollen grains of the apple. The fate of the additional nuclei in the young microspores has not definitely been determined, but failure to observe their presence in the mature pollen grain suggests the possibility that they degenerate.

Degeneration of pollen grains

Observation of considerable material indicates that the relative number of normal liberated microspores present in the loculi of anthers of Stayman Winesap is less than in Delicious. This is probably due in part to degeneration of microspores after formation.

Figs. 28 and 29 show tetrads of Stayman Winesap in which nuclei have degenerated in one and two microspores. In such cases, the disorganized chromatin may partially or entirely fill up the por-

tion of the cell formerly occupied by the normal nucleus. When the former condition occurs, the belief that degeneration has taken place seems justified from the evidence. Regarding the latter condition, however, as in fig. 29, such a belief can with difficulty be sustained. The fact that darkly staining masses have sometimes occurred in all four microspores of Delicious, where it was known that the fixation was poor, leads to the opinion that this effect may not be true degeneration. It has been stated previously that Bouin's modified solution has sometimes given poor results at the synapsis stage. It is also probable that the darkly staining masses in two of the microspores (fig. 29) may be due to the killing and fixing solution, or to some difficulty encountered in the many steps through which the material passes in the preparation of permanent mounts. It is difficult to conceive, however, why one or two of the microspore nuclei of a single tetrad should be in such different stages, or be less permeable to the fixative than those which do not show the abnormal nuclear material.

In stages prior to the pollen grain, chromosome behavior in Delicious and Stayman Winesap has been shown to be markedly contrasting. There now remains the matter of associating chromosome behavior with pollen grain abnormalities which are usually accompanied by peculiarities in the cytoplasm, as well as in the chromatin. In contrast to normal development, such abnormalities have to do with the elimination of hereditary material. In other words, factors carried in the chromosomes may be eliminated by suppression in the pollen grain, or they may be carried through this stage.

Dorsey (11) found that sterile pollen in the grape resulted from degeneration processes in the generative nucleus or arrested development previous to mitosis in the microspore nucleus. He showed in the grape that where degeneration took place soon after the division of the microspore nucleus, both the generative and vegetative nuclei might be affected. If the generative cell was well organized before disintegration began, the vegetative nucleus might remain normal. Valleau (40) stated that in the strawberry there was no specific time at which degeneration of the grains within a single anther took place. In most of the sterile forms studied by him, a series of stages

of degeneration appeared from the first period of growth of the microspore to the formation of nearly mature pollen. DORSEY (12) found the earliest evidence of suppression in the plum immediately following the heterotypic division in an extreme hybrid. In other varieties of plum, suppression began after microspore liberation from the tetrad wall. He found suppression taking place at all stages up to maturity of the pollen grain, and was of the opinion that there were no outstanding conditions which would justify placing the beginning of degeneration in the plum earlier than the period of the dyad nuclei, and degeneration at this stage was exceedingly rare. Pollen development in the plum was found by him to proceed through the heterotypic and homotypic divisions with every appearance of being normal. This condition obtained for the most part in the varieties of pure species, as well as in extreme hybrid forms. DORSEY stated that degenerative processes which became so active later did not gain expression as early as this. He believed, however, that the condition found at nuclear reorganization following the heterotypic division justified the conclusion that degenerative processes might begin earlier in the plum than in *Vitis* or *Fragaria*. In the potato, DORSEY (13), BREEZE (8), and YOUNG (45) agreed that in hereditary sterility degeneration was rapid after tetrads had formed and the pollen grains had assumed their characteristic form. BREEZE stated that irregular heterotypic division seemed responsible for some aborted grains in the potato.

It is possible that in some of the forms where degeneration was not observed until dyad reorganization or tetrad formation, initial causes may have occurred previous to the time their effect became apparent. In Stayman Winesap most of the abnormalities associated with degeneration in the nuclei of the pollen grain are initiated during the heterotypic division stages. It is a well recognized fact that as a rule young cells are less highly vacuolated than old ones. In other words, vacuolation is associated to some extent with degeneration. Pollen grains in which the cytoplasm has become markedly vacuolated before complete degeneration of the vegetative or generative nuclei are found in Stayman Winesap. The normal metabolism of cytoplasm is associated with normal development of chromatin. While vacuolation in the pollen grain may not be of as much signifi-

cance as nuclear degeneration, both processes occur more frequently in Stayman Winesap than in Delicious.

The illustrations considered in this paragraph all refer to Stayman Winesap. In fig. 35 the vegetative nucleus has become large and lengthened. The generative nucleus has also extended. Vacuolation has occurred in the cytoplasm. Fig. 36 illustrates an elongated pollen grain in which vacuolation has taken place in the central portion. The vegetative nucleus is large, the nuclear wall thickened, and the plasma contents massed and disorganized. The chromatin in the generative nucleus appears very small. The pollen grain in fig. 37 has become highly vacuolated in one portion, and both nuclei have gathered toward one end. Fig. 39 represents an irregular shaped pollen grain in which vacuolation is evident, and in which the generative nucleus lies in one of the lobes. Fig. 40 shows a pollen grain where the vegetative nucleus and generative nucleus are large. Considerable vacuolation has occurred, and the nuclear contents have become appreciably disorganized. In all these illustrations it is evident that vacuolation has occurred in the pollen grain before complete degeneration of either the vegetative or generative nuclei. Such features are characteristic of one type of abnormality found in the pollen grains of Stayman Winesap. On the other hand, nuclear degeneration may begin in the pollen grain of Stayman Winesap before the cytoplasm becomes excessively vacuolated. In fig. 41 a pollen grain of Stayman Winesap is shown in which the cytoplasm appears relatively normal, but the generative nucleus has degenerated. Degeneration of this nature may occur in either one of the nuclei, or in both. It may occur at different times in the two nuclei, or simultaneously. Such degeneration seems to be associated with irregular chromosome behavior in previous stages.

Variability in size of pollen grains of different varieties

A preliminary examination of the mature pollen of Delicious and Stayman Winesap indicated that there were marked differences in size and shape between the grains of the two varieties. Size measurements were then made of pollen grains of these varieties and Paragon, which is a member of the Winesap group. In table III is given the frequency distribution, based on diameter measurements of 1000 pollen grains of Delicious, Paragon, and Stayman Winesap.

The frequency data indicate that the mean is much lower in Stayman Winesap and Paragon than in Delicious. BEAUMONT and KNIGHT (5) found that abnormally large and small grains failed to germinate. It will be noted that there are relatively few of these types in Delicious, and that the pollen of this variety is distinctive for the large number of medium sized grains. On the contrary, in Paragon and Stayman Winesap the number of medium sized grains is decidedly smaller. These two varieties are characterized by a great number of small grains and a number of large ones. In regard to the size of pollen grains, significance may perhaps be attached to the works of SAX (35) and KIHARA (23) on the relation of chromosome number to the size of the pollen grain. They believed that the size of the pollen grain of wheat depended on the chromosome number; the greater the number the greater the size. KIHARA also found that dwarf pollen grains are often produced from lagging chromosomes which failed to reach the poles during homotypic division. These small pollen grains were incapable of germination. Large pollen grains which resulted from failure of homotypic division to take place were also observed by him. The reason for the relatively greater frequency of large pollen grains in Stayman Winesap and Paragon than in Delicious has not been determined definitely. It seems probable, however, that the abnormally large pollen grains found in Stayman Winesap and Paragon, like the small ones, are a result of irregular chromosome distribution during meiotic divisions.

TABLE III

FREQUENCY DISTRIBUTION BASED ON DIAMETER MEASUREMENTS OF 1000 POLLEN GRAINS OF DELICIOUS, PARAGON, AND STAYMAN WINESAP

VARIETY	CLASS SIZE OF POLLEN GRAINS IN MICRONS									
	14.9	19.9	24.9	29.9	34.9	39.9	44.9	49.9	54.9	59.9
Delicious	.....	1	6	15	10	100	621	229	18	.....
Paragon	.....	8	29	71	119	242	330	159	37	5
Stayman Winesap	6	8	12	104	172	115	273	241	61	8

Conclusions

The following conditions were thought to exist in the Winesap group of apple varieties when the present investigation was under-

taken: (1) there was almost complete self and inter-sterility; (2) varieties of the group when used as pollen parents in controlled crossing had consistently given a low set of fruit; (3) pollen germination of the members was low; and (4) non-viability was associated in some degree with size of pollen grain.

From a study of a member of the Winesap group, Stayman Winesap, it is found that the chromosomes in the pollen mother cells occur as bivalents and univalents, and that they are paired and distributed irregularly. During meiotic divisions some of the chromosomes lag on the spindle, are extruded into the cytoplasm where they may become organized as small nuclei, and are left outside the daughter nuclei when these form. This irregular behavior of the chromosomes leads to extra microspores and additional nuclei in some microspores, is reflected in abnormal size and shape of the pollen grains, and results in a high degree of pollen sterility. Thus in Stayman Winesap pollen sterility, irregular chromosome behavior, polyspory, and polycary exist. Such conditions are considered to be associated with hybrid forms.

Summary

1. Pollen development in Delicious is normal, while in Stayman Winesap it is characterized by certain abnormalities.

2. Instead of the 14 pairs of chromosomes found in Delicious, there are a number of bivalents and univalents in Stayman Winesap which are irregularly arranged at diakinesis.

3. During heterotypic division in Stayman Winesap some of the chromosomes lag on the spindle and are left in the cytoplasm when the daughter nuclei form.

4. Counts were made which indicate that more than 28 chromosomes are present in the dyad cell of Stayman Winesap.

5. The coarsely knotted, loosely arranged chromosomes resembling spiremes with chromatin extending from the equatorial plate at early metaphase are characteristic of Stayman Winesap during homotypic division. Sometimes only one well defined daughter nucleus seems to be formed. Chromosomes also appear in the cytoplasm during the second division and become organized into small nuclei.

6. In Stayman Winesap more than the usual number of four microspores are frequently found within the tetrad wall (polyspory).

7. Additional small nuclei are sometimes observed in Stayman Winesap in the microspores (polycary); these have never been seen in the later stage of the mature pollen grain.

8. The relative number of normal microspores in the loculi of the anthers is less in Stayman Winesap than in Delicious; thus there is a reduction in the quantity of pollen produced.

9. Degeneration of the pollen grains occurs more frequently in Stayman Winesap than in Delicious. Vacuolation occurs in the cytoplasm of the pollen grain before complete degeneration of the nuclei; or, on the other hand, the nuclei are affected while the cytoplasm is relatively normal. The latter process, which is probably more significant than the former, seems to be associated with irregular chromosome behavior in previous stages.

10. Abnormally small and large pollen grains arise as a result of irregular meiotic divisions.

11. Measurements and grouping of pollen grains indicate that Paragon and Stayman Winesap differ from Delicious in the greater frequency with which small and large pollen grains occur, and in the relative decrease of medium sized grains.

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EXPLANATION OF PLATES XII–XIV

PLATE XII

FIG. 1.—Delicious: relatively early stage of pollen mother cells.

FIG. 2.—Delicious: later pollen mother cells; tapetum separated from pollen mother cells, and middle layers beginning to break down.

FIG. 3.—Delicious: anther tissues when pollen mother cells are in synapsis.

FIG. 4.—Delicious: degeneration of tapetal cells at time of tetrad formation.

FIG. 5.—*Pyrus ioensis* Bailey: degeneration of tapetal cells during homotypic division.

FIG. 6.—Delicious: tapetal cells losing their identity; middle layers elongated in different directions from previous stages; pollen grain stage.

FIG. 7.—Delicious: pollen grain stage; tapetum practically disappeared.

PLATE XIII

FIG. 8.—Delicious: diakinesis.

FIG. 9.—Stayman Winesap: diakinesis; irregular arrangement of chromosomes.

FIG. 10.—Stayman Winesap: diakinesis; chromosomes outside nucleus in cytoplasm.

FIG. 11.—Stayman Winesap: certain manners of pairing of chromosomes.

FIGS. 12, 13.—Delicious: 14 paired chromosomes at metaphase.

FIG. 14.—Delicious: heterotypic anaphase.

FIG. 15.—Delicious: late heterotypic anaphase.

FIG. 16.—Stayman Winesap: interkinesis; large nucleolus and chromosomes in small nuclei in cytoplasm.

FIG. 17.—Stayman Winesap: interkinesis; only one well defined daughter cell formed; chromosomes and nuclei in cytoplasm.

FIG. 18.—Stayman Winesap: interkinesis; bivalents and univalents in cytoplasm; number of chromatin bodies present exceeds number in Delicious.

FIG. 19.—Stayman Winesap: knotted, loosely arranged chromosomes in one nucleus resemble a spireme, and are at early metaphase in other nucleus.

FIG. 20.—Stayman Winesap: homotypic spireme appearance; chromosomes organized into smal nuclei in cytoplasm.

FIG. 21.—Stayman Winesap: homotypic metaphase; strand of chromatin extending from equatorial plate; chromatin in cytoplasm.

FIG. 22.—Stayman Winesap: homotypic telophase; nuclei in cytoplasm.

FIG. 23.—Stayman Winesap: homotypic telophase; large number of nuclei in cytoplasm.

FIG. 24.—Stayman Winesap: homotypic division; metaphase; chromatin seen extending from equatorial plate.

FIG. 25.—Stayman Winesap: dyad reorganization; chromosomes at side of reforming nuclei.

FIG. 26.—Stayman Winesap: homotypic division; chromosomes in cytoplasm.

PLATE XIV

FIG. 27.—Delicious: normal tetrad; only 3 microspores shown.

FIG. 28.—Stayman Winesap: chromatin disorganization in one microspore; additional miniature nucleus in another.

FIG. 29.—Stayman Winesap: disorganization of nuclei in two microspores of a tetrad, probably due to poor fixation.

FIG. 30.—Stayman Winesap: additional nuclei (polycary) in microspores.

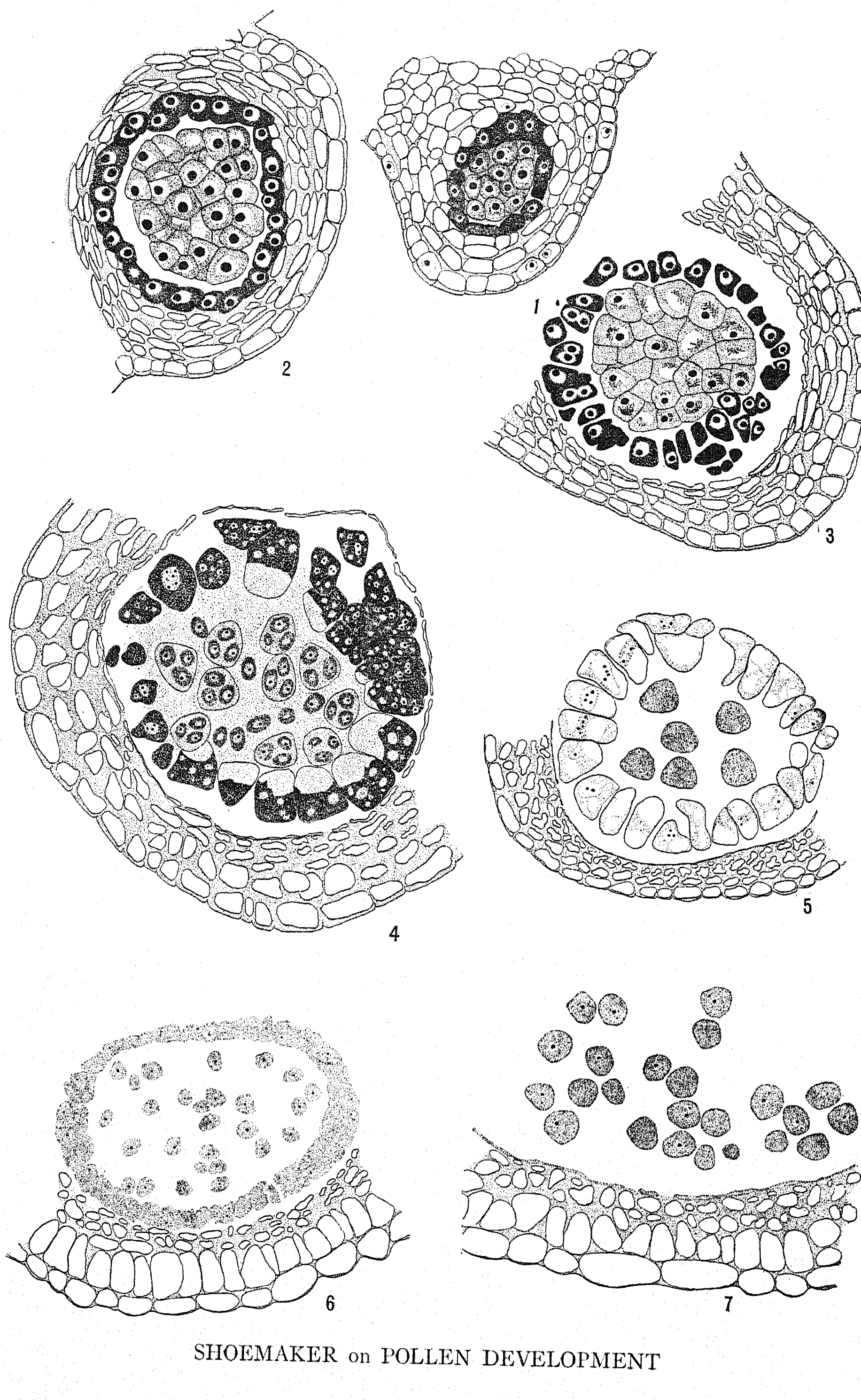
FIG. 31.—Stayman Winesap: three miniature nuclei in a microspore.

FIGS. 32–34.—Stayman Winesap: more than usual number of microspores within tetrad wall (polyspory).

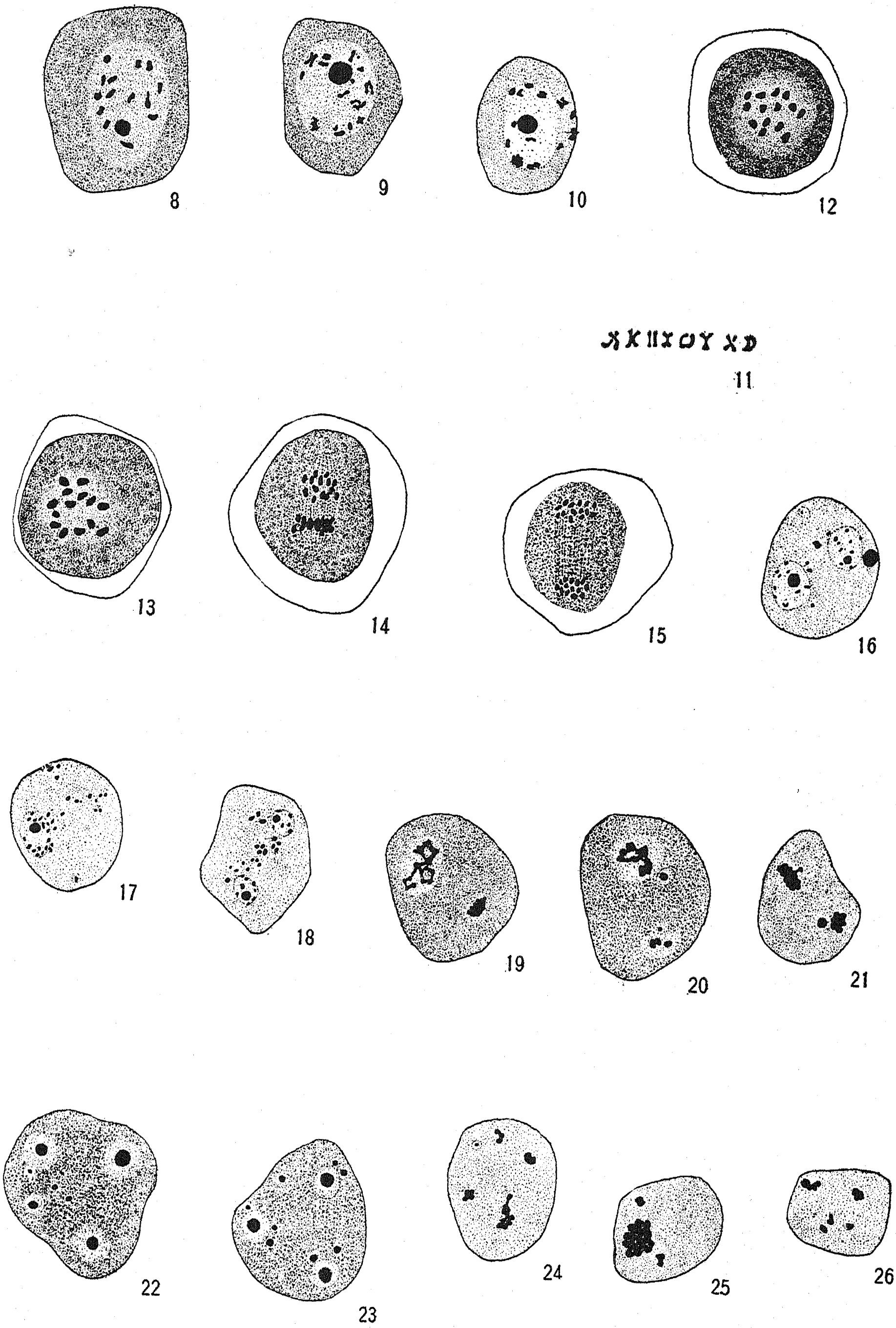
FIGS. 35–40.—Stayman Winesap: nuclear disorganization associated with vacuolation of cytoplasm.

FIG. 38.—Delicious: normal pollen grain.

FIG. 41.—Stayman Winesap: degeneration in generative nucleus of pollen grain before cytoplasm becomes excessively vacuolated.



SHOEMAKER on POLLEN DEVELOPMENT



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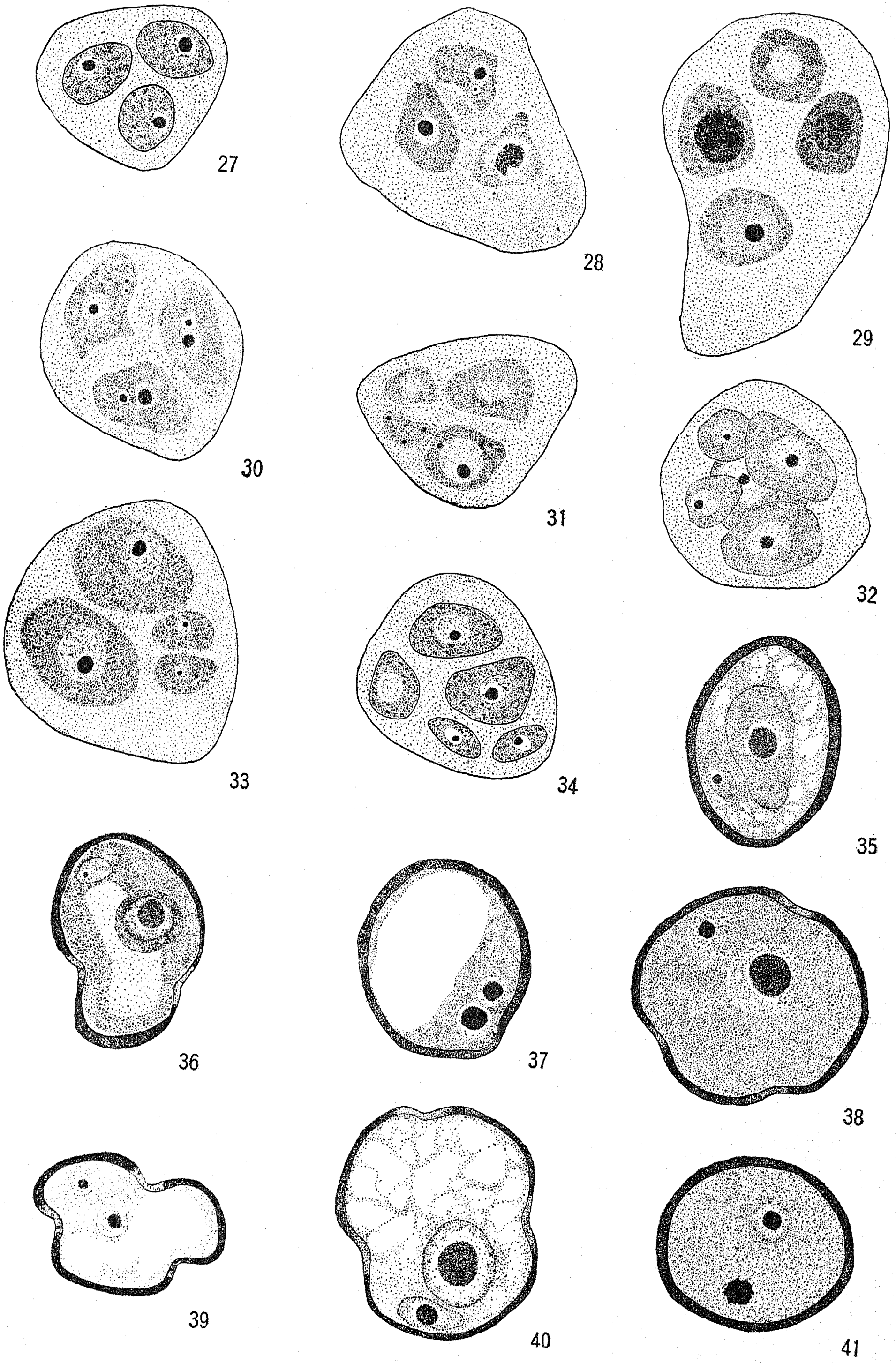

SHOEMAKER on POLLEN DEVELOPMENT

MICROCHEMICAL AND MORPHOLOGICAL STUDIES OF EFFECT OF LIGHT ON PLANTS

NORMA E. PFEIFFER

(WITH PLATE XV AND FOUR FIGURES)

Microchemical studies

In recent years there has developed a tendency to correlate with macrochemical analyses as thorough microchemical studies of the fresh tissues as the investigator can make. By the combination of the two methods, the quantitative results obtained by the one can in some degree be checked, and helpful information as to the distribution and localization of the substances involved can be contributed by the other.

There has been slight evidence of this tendency in investigations dealing with the effect of light duration on plants. The earliest work in which artificial illumination was used (MANGON 19, PRILLEUX 23) served to establish production and activity of chlorophyll as in sunlight. There followed a number of investigations in which artificial light of one sort or another, in varying intensities, was used to supplement daylight. In the major portion of this work, possibly because of commercial importance, much attention was given to the effect on the form, size, weight, time of flowering, and seed production of the plant (ADAMS 1, 2, 3, BAILEY 5, CORBETT 7, DEHÉRAIN 9, HARVEY 15, OAKLEY and WESTOVER 22, SIEMENS 24, TIEDJENS 25, WANN 26, WANSER 27). In relatively few investigations (GARNER and ALLARD 12, 13, LUBIMENKO and SŽEGLOFF 18, NIGHTINGALE 21) the chemical background for these external changes was touched upon. TIEDJENS (25) briefly tested for carbohydrates, and NIGHTINGALE (21) especially supported his macroanalyses with microchemical data.

It seemed important for more complete knowledge of the situation to apply both macrochemical (ARTHUR 4) and microchemical methods to the situations occurring in plants grown in different daily periods.

Conditions of present experiment

The light conditions varied in intensity and amount in the different sets. In one room plants were grown in artificial light supplied by twenty-five 1500-watt lamps, with an intensity varying from 780 foot candles at the beginning of the run to 352 foot candles at the end, as measured by the Macbeth Illuminometer.[1] As determined by the pyrheliometer, the measurement at the beginning was 2.406 gm. calories per sq. cm. per minute, and 1.938 gm. calories at the end. The daily periods of illumination were for 5, 7, 12, 17, 19, and 24 hours for the different sets of plants in this room. There was a fluctuating supply of carbon dioxide, averaging 0.3 per cent. The temperature was constant at 25.5° C., and the humidity at 80 per cent of saturation.

Plants in two houses equipped with a gantry crane[2] carrying forty-eight 1000-watt lamps, had 6 hours of additional illumination beyond natural daylight. In one case this artificial light was supplied from 6:00 P.M. to midnight, in the other from midnight to 6:00 A.M. The former had an extra supply of carbon dioxide, the latter the usual atmosphere. In both cases the artificial light was 383.69 foot candles (Macbeth Illuminometer) and 0.465 gm. calories per sq. cm. per minute (pyrheliometer) at the beginning of the experiment, and gradually diminished to 141.5 foot candles (Macbeth Illuminometer) and 0.323 gm. calories (pyrheliometer) at the end. A house with ordinary atmosphere and the natural light conditions of the season (March, April, and beginning of May) served for the control plants. The temperature and humidity were as in the continuous light room.

Methods

From previous experience it was deemed that an intensive study of the tomato (*Lycopersicum esculentum*), which shows a decided reaction to longer periods of light, would be of importance. Studies were made of individual plants of the variety Bonny Best in each of the series in different daily periods of illumination, at various in-

[1] Data supplied by J. M. ARTHUR.

[2] Greenhouses equipped with gantry crane are described and illustrated in Contr. Boyce Thompson Inst. Plant Research 1:17. figs. 5, 6. 1925.

TABLE I

TOMATO; MICROCHEMICAL ANALYSES

HOURS LIGHT	AGE (DAYS)	SUGARS	STARCH	BIURET	NITRATES	PHOSPHATES	MAGNESIUM	GENERAL RATIO
5	7	Very little	Very little	Good	Very high	....	....	Low carbohydrates: low protein
	15	Very little	Very little	Little	Much	....	....	
	25	Very little	Very little	Little	Excessive	....	....	
	43	Increase	Very little	Little	Excessive	Negative	Negative	
7	7	Very little	Very little	Little	Very high	....	....	Low carbohydrates: low protein
	16	Little	Little	Little	Much	....	....	
	28	Little	Little	Little	Excessive	....	....	
	44	Little	Little	Little	Excessive	Very little	Negative	
12	6	Little	Moderate	Little+	Very high	....	....	Slightly increased carbohydrates: slightly increased protein
	16	Little	Decrease	Little+	Much	....	....	
	25	Little	Decrease	Negative	Excessive	....	....	
	44	Increase	Little	Little	Moderate	Negative	Negative or trace	
17	8	Little	Little	Moderate	Very much	....	....	Higher carbohydrates: protein as in 12 hours
	16	Little	Little	Moderate	Much	....	....	
	24	Increase	Increase	Little	Excessive	....	....	
	38	Greater increase	Increase	Little	Moderate or negative	Very little	Trace to fair	
19	8	Little	Little	Moderate	Very much	....	....	Low carbohydrates: low protein
	16	Slight increase	Little	Little	Much	....	....	
	24	Same	Little	Little	Excessive	....	....	
	37	Increase	Slight increase	Decrease	Excessive	Negative or little	Little to much	
24	9	Little	Little	Moderate	Excessive	....	....	Slightly increased carbohydrates: higher protein
	17	Little	Little	Little+	Very much	....	....	
	25	Little	Little	Little+	Excessive	....	....	
	48	Great increase	Moderate	Little to good	Negative	Negative or little	Fair	
Control	7	Negative	Little	Good	Excessive	....	....	Moderate carbohydrates: lower protein
	18	Very little	Fair+	Fair	Excessive	....	....	
	27	Increase	Fair+	Little	Excessive	....	....	
	45	Same	Moderate	Decrease	Negative	Negative or trace	Moderate	
Gantry crane house, $+CO_2$	7	Very little	Very little	Little+	Excessive	....	....	Moderate carbohydrates: low protein
	16	Fair	Fair	Little+	Excessive	....	....	
	35	Moderate	Moderate	Little+	Negative	Fair	Moderate	
Gantry crane house, usual atmosphere	8	Negative	Little	Fair	Excessive	....	....	Moderate carbohydrates: less protein
	16	Fair	Little+	Little	Excessive	....	....	
	35	Moderate	Fair	Very little to little	Trace	Negative	Much	

tervals, until the plants were about 6 weeks old. Analyses were made at as near the end of the daily exposures to light as possible. Tests were made for the carbohydrates, protein, nitrates, magnesium, calcium, and phosphorus, on free-hand sections in each of 5 regions of the plant, namely, root, lower stem, middle stem, upper stem, and leaf, with the object of obtaining an idea of the plant chemistry in different regions. Aside from this intensive study of a single form, buckwheat (*Fagopyrum esculentum*) of Henderson's Japanese variety was considered, with a longer interval between analyses, and a few other forms were examined for supplementary data at but one time. Unfortunately, in this phase of the work there is some error due to individual differences, since there were not enough plants of a single kind to avoid this difficulty.

Standard tests were used, Flückiger's reaction for fructose, glucose, and dextrin; potassium iodide solution of iodine for starch; a similar solution and biuret test for proteins; diphenylamine in 75 per cent sulphuric acid for nitrates; formation of ammonium magnesium phosphate crystals for magnesium and phosphorus, by use of appropriate reagents in each case; and 5 per cent sulphuric acid for calcium.

Results

In the case of tomato, the results of tests, made at four intervals until the plants were about 6 weeks old, can be shown best in tabular form (table I), giving the general conclusion as to the content of the plant from a study of all the regions. This table does not take into account the amount of tissue development, which is more properly considered under the anatomical discussion. Similar tables bring out the important points in regard to buckwheat (table II), as determined at two stages, when the plants were about two and a half weeks old and 7 weeks old. In four o'clock (*Mirabilis Jalapa*) (table III) the aerial part only was tested when the plants were about 5 weeks old. At the conclusion of the experiment, when about 10 weeks old, the roots were examined. The table dealing with the earlier tests shows the part of the body commonly used for gross analyses; the latter, dealing with the organ commonly overlooked, gives interesting data in regard to reserves in different light durations (see also morphology). Observations on other plants, although too scat-

TABLE II

BUCKWHEAT; MICROCHEMICAL ANALYSES

HOURS LIGHT	APPROXIMATE AGE (DAYS)	SUGARS	STARCH	BIURET	NITRATES	PHOSPHATES	MAGNESIUM	GENERAL RATIO
5	17 48	Little Decrease	Little Increase	Fair Little	Much Excessive	.... Negative	.... Trace	Low carbohydrates: low protein
7	17 48	Little Decrease	Moderate Increase	Little+ Decrease	Much Excessive	.... Excessive	.... Excessive	Low carbohydrates: low protein
12	17 48	Little Decrease	Fair Increase	Moderate Little	Very much Excessive	.... Much	.... Excessive	Low carbohydrates: low protein
17	17 48	Little Increase	Moderate Increase	Fair Little	Much Negative	.... Trace	.... Little	High carbohydrates: low protein
19	17 48	Little+ Increase	Moderate+ Increase	Fair Little−	Much Negative	.... Moderate	.... Much	High carbohydrates: low protein
24	17 48	Little+ Slight increase	Little+ Good increase	Fair Fair	Much Trace	.... Fair	.... Very much	High carbohydrates: more protein (than 17 or 19 hours)
Control	17 48	Little+ Decrease	Little+ Decrease	Moderate Little	Much Excessive	.... Much	.... Moderate+	Low carbohydrates: low protein
Gantry crane house, $+CO_2$	17 48	Little+ Increase	Fair Increase	Fair Decrease	Much Trace	.... Much	.... Little	High carbohydrates: low protein
Gantry crane house, usual atmosphere	17 48	Little− Very slight increase	Fair Slight decrease	Fair Slight decrease	Much Fair	.... Fair	.... Fair+	High carbohydrates: fair protein

TABLE III

FOUR O'CLOCK; MICROCHEMICAL ANALYSES

Hours light	Approximate age (weeks)	Sugars	Starch	Biuret	Nitrates	Phosphates	Magnesium	General ratio
				Aerial parts				
5	5	Very little	Little	Much	Excessive	Little	Little	Low carbohydrates: high protein
7	5	Little	Little	Much	Very much	Very little	Moderate	Low carbohydrates: high protein
12	5	Slight increase	Slight increase	Much	Excessive	Negative	Much	Low carbohydrates: high protein
17	5	As above	As above	Slight increase	Excessive	Much	Very much	Slightly more carbohydrates: high protein
19	5	Fair	Little	Moderate	Excessive	Much	Very much	Slightly more carbohydrates: high protein
24	5	Moderate	Little	Fair	Excessive	Negative	Moderate	Higher carbohydrates: low protein
Control	5	Very little	Little	Moderate+	Excessive	Very little	Moderate	Low carbohydrates: high protein
Gantry crane house, $+CO_2$	5	Fair	Fair+	Moderate+	Excessive	Negative	Moderate+	Higher carbohydrates: high protein
Gantry crane house, usual atmosphere	5	Moderate	Moderate	Moderate+	Excessive	Negative	Moderate	Higher carbohydrates: high protein
				Roots				
5	10	Negative	Moderate	Fair	Excessive	Negative	Negative	Low carbohydrates: low protein
7	10	Negative	Much	Fair	Excessive	Negative	Negative	Low carbohydrates: low protein
12	10	Very little	Much	Fair−	Excessive	Trace	Negative	Higher carbohydrates: low protein
17	10	Very little	Very much	Fair	Fair	Moderate	Fair	High carbohydrates: low protein
19	10	Little	Excessive	Fair	Fair	Fair	Little	High carbohydrates: low protein
24	10	Trace	Excessive	Fair	Little	Much	Much	High carbohydrates: low protein
Control	10	Negative	Much	Fair−	Very much	Fair	Much	Less high carbohydrates: low protein
Gantry crane house, $+CO_2$	10	Very little	Excessive	Fair+	Fair−	Much	Much	High carbohydrates: low protein
Gantry crane house, usual atmosphere	10	Little	Excessive	Fair−	Very much	Fair	Much	High carbohydrates: low protein

tered to be worthy of tabulation, are depended upon in the following discussion.

Discussion

In coordinating the facts observed, one finds that usually in the short exposures to light there is a low carbohydrate and low protein content, which is coupled, as may be seen in the anatomical work, with relatively less tissue production than in longer exposures. The most marked exception to this ratio is seen in the four o'clock, where the aerial parts early show a relatively higher protein content. In the longer exposures, under similar atmospheric conditions, there is evident a tendency toward greater production of carbohydrates without a proportionately increased ability to utilize the manufactured product in protein formation and tissue development. In tomato and four o'clock this increase, evident in the 12-hour exposure, is even clearer in 17 hours of light. The tomato suffers injury, however, in this and higher durations, with the result that photosynthetic ability falls markedly, so that in 19 hours the carbohydrates are again relatively low. A comparable fall does not appear in the buckwheat, which seems better able to utilize longer exposures. In all cases, except possibly four o'clock, there is a greater carbohydrate content in the plants in the gantry crane house with extra carbon dioxide than in those with the ordinary atmosphere.

In comparisons of the nitrates present in the different sets of conditions in different plants, variation is evident at once. The tomato in longer exposures, as in shorter, shows great amounts of nitrate present. Only in the control and in the two houses receiving extra light beyond daylight are there negative results or only traces. In four o'clock, on the other hand, there are ample nitrates in the aerial part in all series, although the subterranean parts show much less at the end of the run in 17, 19, and especially in 24 hours' exposures. Practically the same thing is true in buckwheat as a whole as in four o'clock roots. In other forms examined, it was found that usually the nitrates ran lowest in the gantry house with extra carbon dioxide. There is a possibility that the plants in this series, making the great growth that they do, draw too heavily on the soil, and may not have adequate available nitrates at the end of the run. This point will be guarded more carefully in further work. It may be

pointed out, however, that such low nitrate content in these plants was found at various stages in several plants, and so was not usually due to low soil supply.

Data in regard to the occurrence of phosphates and magnesium are as yet inadequate for drawing general conclusions. Both appear relatively lower as a rule in the shorter exposures, due probably to proportionately greater utilization in production of proteins and tissue formation.

Anatomical studies

Literature

The most comprehensive study of the anatomical differences brought about by artificial light was made by Bonnier (6). Using arc lights as continuous light or for periods of 6 hours in the morning and 6 hours in the afternoon, with fairly constant temperature and humidity, he grew a wide variety of forms and made studies of stem and leaf characters. He drew the following conclusions in regard to the effect of continuous light: (1) the chlorophyll is greater in amount and more uniformly distributed in all the cells containing it in the normal plant; (2) the leaf blade structure is simplified, that is, the palisade is less distinct or entirely lacking and the epidermis has thinner walls; (3) the stem structure is simplified, with late or poorly developed cork, less distinct endodermis, less lignification and sclerification. He also found that the structures in discontinuous electric light were more like those in discontinuous solar light than those in continuous electric illumination. He thinks of continuous light as producing a result much like etiolation except for greater greenness.

Lubimenko and Szégloff (18), dealing with periods of light from 14 hours down to 4, found greater concentration of chlorophyll in the longer exposure, by spectrocolorometric methods rather than by chloroplast study.

More recently, Massart (20) studied the effect of continuous light on leaf structure. With a range of forms including representatives from Hepaticae, *Selaginella*, and flowering plants, he used intervals of 6, 12, 18, and 24 hours' exposure to light. He comes to the conclusion that effects on form and structure are more dependent on

intensity than on duration, and that in the intervals used, continuous and discontinuous light affect assimilatory organs in the same way.

Miss DEATS (8), in her anatomical studies of pepper and tomato grown in intervals of 6.5 hours (short day), 17.5 hours (long day), and the normal day period, found that the amounts of bast and xylem in stems varied directly with the length of day. There was variation in the thickness of their cell walls in the same direction. Similarly the size of the epidermal cells and the amount of cork varied directly with the duration of light. In leaves, the greatest thickness and size and depth of greenness occurred in long day plants, the least in short day.

RESULTS

STEM STRUCTURE.—An attempt was made to follow the degree of development of the stem whenever sections were made for microchemical studies. Inasmuch as only a single specimen was used each time, there is bound to be individual variation in the results. The plants were not uniform in development, and one must be aware of this fact.

It seemed that the structure near the base of the stem is the most conservative that one could use for demonstration of results, since the region used could be more exactly determined as a definite distance from soil surface. "Middle stem" is less exact, since plants attained different heights in different light intervals, and the "middle" would be at various levels. Similarly the tip of the stem and the roots, which varied from plant to plant, seem to add less to our knowledge of how the light affected the plant than the base of the stem. I therefore made diagrams of this region in tomato (text figs. 1, 2) and buckwheat (text fig. 3), to show the relative amounts of tissue developed in the different sets of conditions. In tomato these correspond roughly to each other in the different series, sometimes with variation of several days between comparable examinations. This variation and individual variation must both be considered in appreciating how well such diagrams portray the actual effect. Text figs. 1 and 2 show the stems at the time of the first, third, and fourth microchemical analyses (compare table I) in all series up to 24 hours' illumination; of the first, second, and third in the gantry crane

houses; and first, second, and fourth in the control set. It should be borne in mind, therefore, that the second diagram of the control shows a younger plant (7 days' difference) than most of the series,

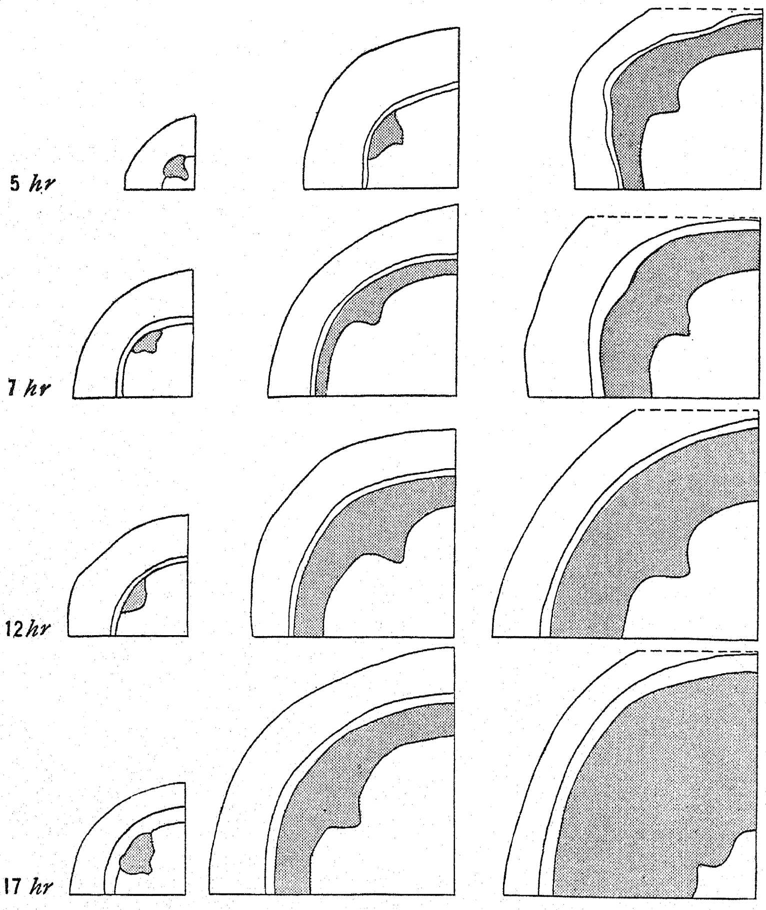

FIG. 1.—Diagrams of bases of stems of tomatoes grown in daily light durations of 5, 7, 12, and 17 hours, showing proportions of tissues at time of first, third, and fourth microchemical analyses in columns 1, 2, and 3 respectively; xylem shaded; compare table I.

and that the two gantry crane house plants were not so old in their last pictured stage as all other series.

In buckwheat there is better correspondence in the stages, since only two analyses were made, with diagrams at these stages.

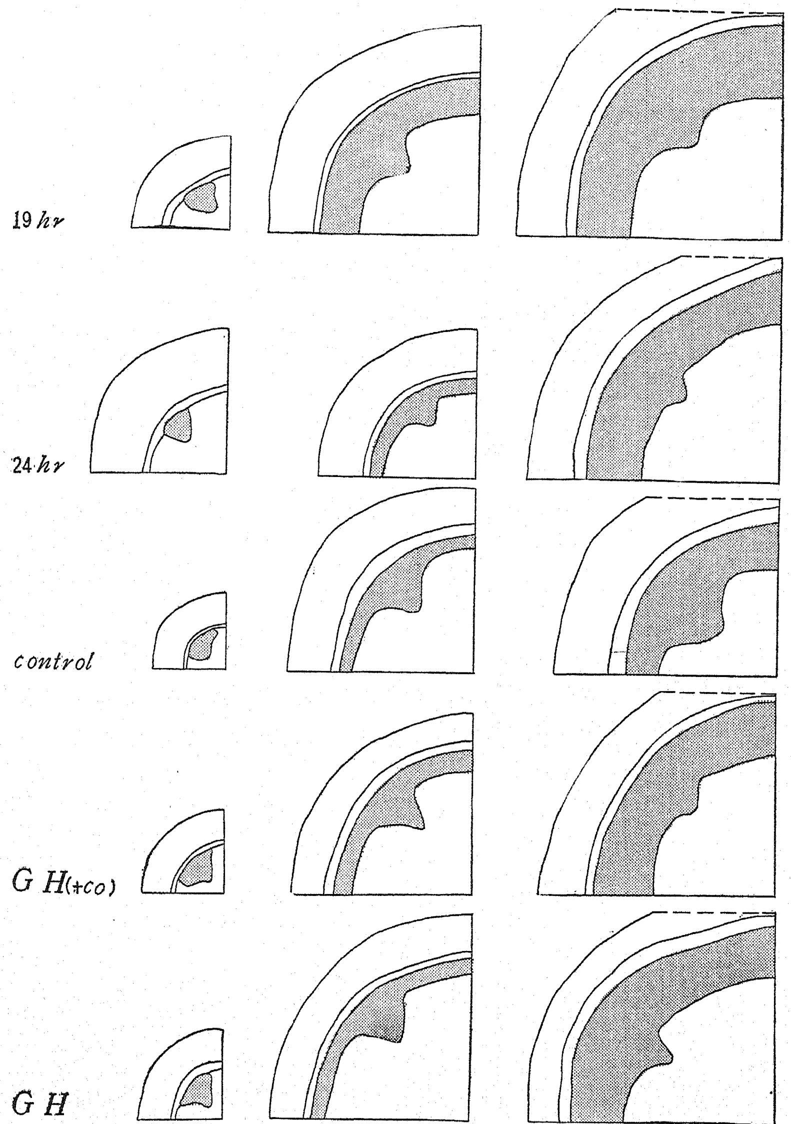

FIG. 2.—Diagrams of bases of stems of tomatoes grown in daily light durations of 19 and 24 hours, in control house (normal light of season), and in gantry crane houses with extra carbon dioxide and usual atmosphere; first, second, and third columns correspond, in 19 and 24 hour sets, to first, third, and fourth microchemical analyses respectively; in control, to first, second, and fourth microchemical analyses; in gantry crane houses, to first, second, and third microchemical analyses; compare table I.

In tomato all series with light duration of 12 hours or more attain as great a diameter or greater than the control, regardless of the

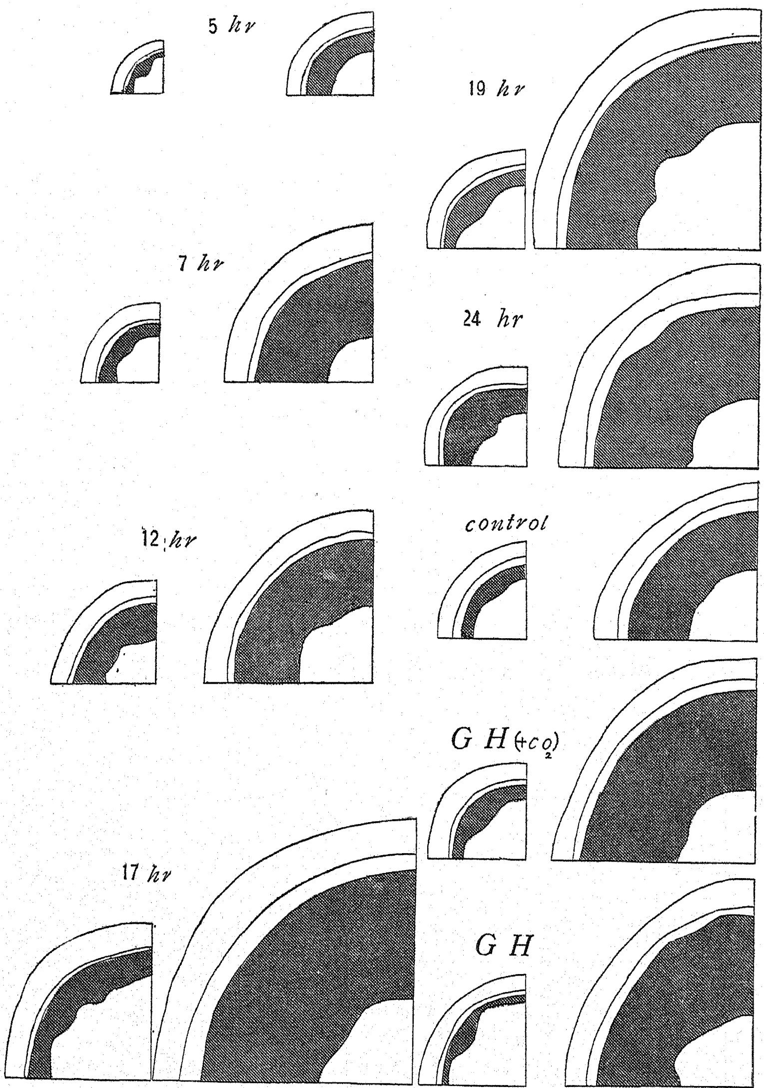

FIG. 3.—Diagrams of bases of stems of buckwheat in different sets of experiment: in each couple of diagrams the smaller shows condition at 17 days, larger at 48 days; compare table II with microchemical data.

success of the plant in other respects; but in several cases, as the 12 and 17-hour plants, there is a very pronounced development of xylem tissue, with relatively less cortex and pith contributing to the diameter. The 12-hour plant was rather diffuse in habit, with somewhat yellow-green leaves, and attained the greatest height of any series. Correlated with the chemical data, this series, having as reserves more carbohydrates and more protein than the lower series, was also utilizing more of these in upward growth than any other set, and gave good evidence in cross-sections of their utilization in highly differentiated rather than simple tissues.

The 17-hour plant, in comparison, although evidently producing much differentiated tissue, was only about three-fourths as tall, had lost all its lower leaves up to the seventh internode, and had a decidedly yellow aspect in the small bladed leaves remaining. It was evidently suffering, as were the plants with longer light durations, from the conditions under which it grew. At the time of cessation of the experiment it showed more carbohydrate reserve, but less protein than the 12-hour form. Considering the condition of the leaves, it is entirely likely that the plant would soon have reached the stage which the continuous light plant attained at about the termination of the run, and succumbed. The 19-hour and continuous light plants were successively shorter than the 17-hour, with progressively poorer physical appearance, as evinced by loss of leaves, yellowing, and, in the case of the 24-hour exposure, by the production of brown areas on leaves, petioles, and stems. Analyzed, these patches (fig. 20) show a sort of cork structure; sometimes similar brown patches are found inside the stem tissues. These give a cellulose reaction when tested, and fail to show tannins.

The gantry crane house plants showed somewhat similar results in tissue development when compared with one another, but the plants supplied with extra carbon dioxide were taller than the others. Compared microchemically, the latter had less reserve protein than the former, and both had only moderate reserve carbohydrates.

As might be expected, the plants with only 5 or 7 hours of light were smaller, very open in habit, with small blades as compared with the total size of the leaves. They were also very watery, and the thin leaves wilted readily. There was little development of the character-

istic complex oil that gives the plant its odor, in these or in the plants with 17, 19, or 24 hours of light.

In buckwheat the maximum diameter was attained in the plants grown in 17 hours of light. These obviously developed the greatest amount of xylem, and showed the greatest height, except for the plant in the gantry house with the usual atmosphere, which made it a close second. The shorter exposures produced correspondingly shorter plants, and correspondingly less of the highly differentiated tissues. Longer exposures show a similar succession from this maximum, although in less degree. The 19-hour and continuous light plants are somewhat shorter than the 17-hour, but almost twice as tall as the 7-hour plant. As compared with one another, there was a slight difference in the 19 and 24-hour plants in favor of the latter as regards both height and amount of xylem, which I believe to be due to variation in individuals rather than to direct effect of light.

The gantry crane house plants showed a slightly better development of xylem and bast when provided with extra carbon dioxide. On comparison with the rest of the series, they show less development than the 17-hour plant, and correspond fairly well with those receiving 19 or 24 hours of light.

In relating these facts with the microchemical data, the 5, 7, and 12-hour plants, like the control, showed low carbohydrate and low protein reserves, while those in longer exposures showed much carbohydrate and low protein. At the time of the last analysis all plants were in flower, but only those in 7 and 12 hours had set fruit. It is clear that the buckwheat attains maximum height and development of tissues and reserves in 17 hours' light exposure, in contrast with tomato, which, although attaining maximum tissue differentiation and reserves, fails to make the growth in height and total diameter. In summation of all points observed, the tomato is more successful in 12 hours' exposure.

LEAF STRUCTURE.—*Capsicum annuum* (variety Ruby King), *Coleus* sp., *Glycine Soja* var. Tokyo, Peking, Mandarin, and Biloxi, *Lactuca sativa* var. Mignonette, *Mirabilis Jalapa*, *Nicotiana Tabacum* var. Imported Havana, *Pelargonium* sp., *Solanum Melongena* var. Black Beauty, *Tropaeolum majus* (Grant Flowering Salmon Queen), *Lycopersicum esculentum* var. Bonny Best, and *Viola* sp. were here

considered. In addition, *Brassica oleracea* var. *capitata* (Early Jersey Wakefield) was used in determining the number of stomata.

Measurements of mature leaves in similar positions on the plants were made by means of an ocular micrometer on free-hand sections (table IV). In pepper, coleus, lettuce, tomato, four o'clock, geranium, and nasturtium, continuous light tended to reduce the thickness of the leaf blade to a greater or less degree, as compared with control plants (pl. XV). The effect of increase in length of light period to 19 hours in the gantry crane houses did not produce such uniform results as these. Pepper, coleus, lettuce, tobacco, and three varieties of soy bean (Tokyo, Peking, and Mandarin) showed a decrease in leaf thickness in both houses, whereas geranium, eggplant, nasturtium, tomato, violet, and Biloxi soy bean were thicker leaved in both houses. Four o'clock leaves were evidently thinner in the gantry crane house with ordinary atmosphere, but only very slightly thicker in that supplied with extra carbon dioxide.

When the comparison is made between plants in the two gantry crane houses, it is found that in two cases, nasturtium and Mandarin soy bean, the leaf thickness is the same under the two sets of conditions. In the majority of cases (nine), however, the reduction in leaf thickness is greater in the house where no carbon dioxide is supplied, while in three (tobacco, tomato, and lettuce) the reverse is true. This would tend to show that in the greater number of forms considered, the longer duration favored reduction in leaf thickness, which the increase in carbon dioxide content served to counterbalance in part. The number of forms and the number of individuals considered, however, do not warrant any sweeping generalizations.

In making measurements of leaves, attention was also given to the development and length of the palisade cells (table IV). As one might anticipate, usually there is a correlation, the palisade cells being shortest where the leaf is thinnest, and longest where it is thickest, so that there is shorter palisade in the thinner leaves in continuous light. To this generalization there are occasional exceptions, as the four o'clock and tomato in the gantry crane house with extra carbon dioxide. Obviously, where there is greatest decrease in thickness, the length of cells in the palisade layer may be so reduced that it is little differentiated in size from the adjacent

spongy cells (as coleus in continuous light); or, where a second palisade layer is usually present, this may be lacking (geranium in continuous light), or less marked (Peking soy bean in gantry houses).

TABLE IV

THICKNESS OF LEAVES AND LENGTH OF PALISADE CELLS

		Control (mm.)	Continuous light (mm.)	Gantry crane house, $+CO_2$ (mm.)	Gantry crane house, usual atmosphere (mm.)
Capsicum annuum	Leaf	0.28	0.26	0.22	0.208
	Length	0.12	0.087	0.086	0.086
Coleus sp.	Leaf	0.27	0.14	0.21	0.18
	Palisade	0.06	0.046	0.07	0.049
Glycine Soja var. Biloxi	Leaf	0.35	....	0.45	0.346
	Palisade	0.096	....	0.157	0.14
		0.054	....	0.06	0.06
var. Mandarin	Leaf	0.48	....	0.40	0.40
	Palisade	0.20	....	0.10	0.115
		0.10	....	0.09	0.08
var. Peking	Leaf	0.43	....	0.25	0.21
	Palisade	0.14	....	0.07	0.05
		0.11	....	0.04	0.04
var. Tokyo	Leaf	0.34	....	0.32	0.26
	Palisade	0.085	....	0.085	0.065
		0.09	....	0.06	0.065
Lactuca sativa	Leaf	0.236	0.138	0.18	0.208
Lycopersicum esculentum	Leaf	0.27	0.24	0.35	0.48
	Palisade	0.12	0.08	0.10	0.10
Mirabilis Jalapa	Leaf	0.27	0.26	0.277	0.22
	Palisade	0.095	0.08	0.064	0.05
Nicotiana Tabacum	Leaf	0.28	....	0.22	0.27
	Palisade	0.09	....	0.08	0.065 } double
					0.055 } palisade
Pelargonium	Leaf	0.229	0.225	0.33	0.31
	Palisade	0.05	0.059	0.08	0.08
		0.038	No 2nd palisade	0.06	0.04
Solanum Melongena	Leaf	0.22	....	0.26	0.23
	Palisade	0.08	....	0.16	0.11
Tropaeolum majus	Leaf	0.15	0.13	0.17	0.17
	Palisade	0.047	0.037	0.05	0.05
Viola sp.	Leaf	0.17	....	0.21	0.18
	Palisade	0.07	....	0.09	0.076

In regard to the epidermis, specific measurements were not made. From general observations, no marked trend was noted, nor were marked variations evident in the spongy tissue of leaves under different conditions. In the houses with additional light beyond solar light there was an evident increase in hairiness (soy beans), and a yellow appearance to the leaf rather than the good green of the check

plants. In this latter feature, the plants in the usual atmosphere are intermediate in coloring between the control and those with extra carbon dioxide. Evidently the prolonged light brings about some yellowing, which is aggravated by the increase in carbon dioxide. That there is some difference in size and distribution of chloroplasts is clear even to the casual observer, but the data are inadequate for a clear presentation of these points.

STOMATA.—Counts were made to determine the number of stomata on both leaf surfaces in six forms grown in four different situations. Table V shows the calculations for a square millimeter surface based on the averages from counts of thirty fields. The forms are too few to attach great weight to the results, but the latter may be utilized as a starting point for more thorough studies in later experiments. In continuous light, there are approximately uniform results, usually with reduction in number of stomata on both surfaces (table V). In the gantry crane houses there is sometimes an increase, sometimes a decrease, but usually the results are in the same direction for both houses and for both surfaces. This would seem to show that light is more effective as a factor in determining the number of stomata than is carbon dioxide. The gantry crane houses had similar light conditions, but different in intensity as well as duration, from those in continuous light. There is therefore no good basis of comparison for these two sets.

TABLE V

COUNTS OF STOMATA PER SQ. MM. OF LEAF SURFACE; AVERAGE OF THIRTY FIELDS

	SURFACE	CONTROL	CONTINUOUS LIGHT	GANTRY CRANE HOUSE, $+CO_2$	GANTRY CRANE HOUSE, USUAL ATMOSPHERE
Brassica oleracea var. capitata	Upper	116	87	149	69
	Lower	161	155	207	126
Capsicum annuum	Upper	42	15	60	61
	Lower	113	97	232	187
Lactuca sativa	Upper	27	39	62	99
	Lower	41	77	47	120
Mirabilis Jalapa	Upper	62	12	83	65
	Lower	150	136	152	266
Pelargonium sp.	Upper	26	15	21	44
	Lower	148	67	68	121
Solanum Melongena	Upper	195	....	85	130
	Lower	242	....	204	221

An attempt was made to correlate the number of stomata with thickness of leaves. In the majority of cases where both were determined, there seemed a tendency to an increase in the number of stomata where the leaves were thinner. The number of cases, however, is too small for generalizations.

ROOTS.—Roots of tomato and buckwheat were examined at the time of study of other organs. Although there was not enough material for intensive work, and pot conditions are apt to cause greater variation in root systems than in aerial, nevertheless there was evident correlation with the development of the base of the stem. Since a plant with a fleshy root might give valuable additional data because of storage material, four o'clock roots were examined at the close of the experiment. The short period plants then showed low carbohydrate and low protein reserves and a small volume (as measured by displacement of water). There was a marked increase in volume in 17 and 19-hour plants, which in turn were smaller than the gantry crane house plant with usual atmosphere, the continuous light, and the other gantry crane house plant, which represented the maximum root development. The increase in diameters of roots was roughly comparable with volume, with 0.35 cm. at top for the 5-hour plant and 1.0 cm. for the 7-hour plant. In longer exposures, however, the simple root of low durations gave way to branched, very fleshy systems.

In contrast to the root systems, the aerial part attained the greatest height in 17 hours' exposure, next successively in (1) 19 hours, (2) gantry crane house with increased carbon dioxide, (3) other gantry crane house, and (4) continuous light and 12 hours, which were approximately equal. Text fig. 4 shows the volumes of roots and heights of stems, indicating that a conclusion in regard to the manufacture of materials, based on the stem height alone, would have been very misleading in this form.

DISCUSSION

A comparison of these results with those of previous workers brings out both similarities and contrasts. In leaves, continuous light produces thinner leaves with less pronounced palisade cells as seen by BONNIER (6). This fails to substantiate MASSART'S (20) conclu-

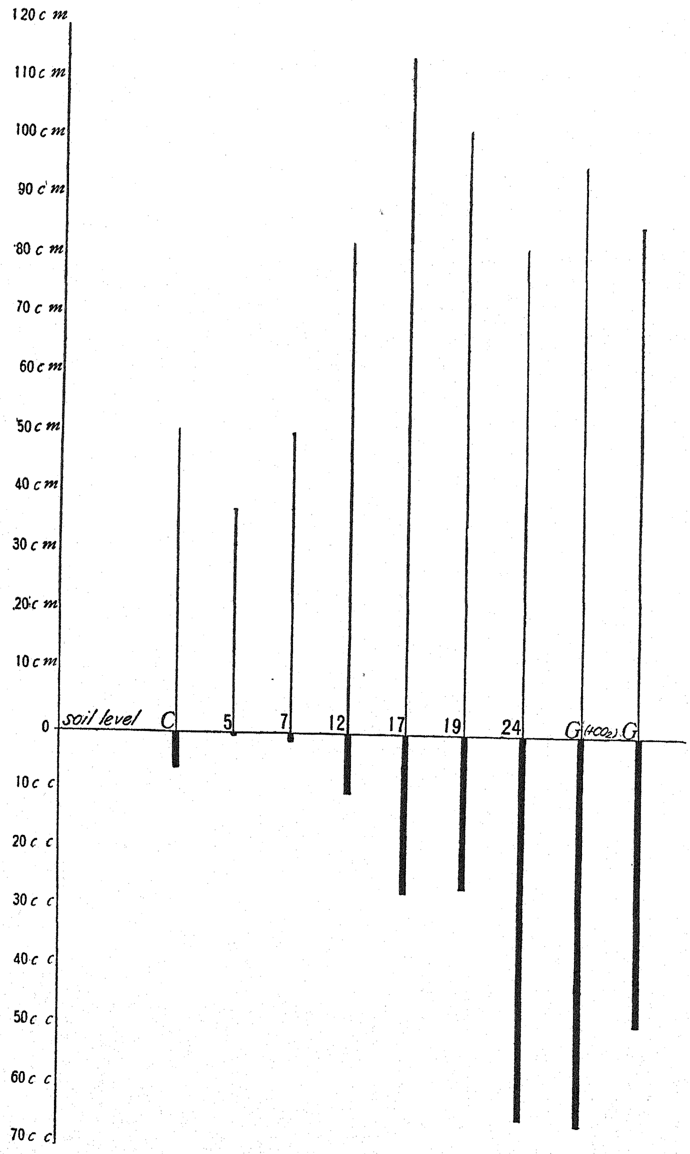

FIG. 4.—Relative development of stem and root regions of four o'clock; lines above "soil level" show height of stem in cm., lines below show volume of roots in cc., successively in control set, in daily light durations of 5, 7, 12, 17, 19, and 24 hours, and in gantry crane house with extra carbon dioxide and with normal atmosphere.

sions in regard to the general similar effect of 24, 18, 12, or indeed 6 hours' illumination on form and structure of leaves. MASSART believes that intensity is more effective in producing structural and form changes, and it may be that differences in intensity are responsible for differences in results obtained by investigators. The earlier records usually include the type of lamp used, range of distances of plants from lamp, but not the measurement of the light falling on the plant.

In long day plants in the gantry crane houses, thinner leaves were produced in the pepper, but thicker in the tomato. The latter is in accord with the work of Miss DEATS (8) on a similar form, the former is not. The presence of extra carbon dioxide might be a factor in one of these houses, but is not active in the other. Further work with more specimens may explain the difference in results.

In regard to stomata, no special work is on record of counts made under similar conditions. A comparison with the results of Miss ECKERSON (11) shows a difference in the control plants, due to conditions of growth in all likelihood. DUFOUR (10) has shown the result of greater intensity of light to be greater number of stomata. In my experiment, the continuous light plant had the lowest light intensity and the lowest number of stomata. Some gantry crane house plants, however, with equal intensity to the control plants during normal daylight plus an additional period of artificial light, also showed reduction in number, tending to remove responsibility from the intensity factor. The matter is complicated by the carbon dioxide supply, which makes an exact comparison with previous work impossible. In correlating with thickness of leaf, I find the usual result in most cases, the larger number of stomata in thinner leaves.

In the stem I failed to find the simplification of tissues reported by BONNIER. There was better agreement with the results of Miss DEATS, who found variation in amount of xylem in short day plants (6.5 hours), normal day plants, and long day (17.5 hours), in direct accord with the length of day. This carries no inference that further increase in time of exposure would further increase the amounts of xylem, under other similar conditions. Indeed my results show for the forms considered that there is a period of exposure for each form, beyond which additional duration is ineffective in producing differ-

entiated tissue, or increased height. The best interval for producing differentiation and height in tomato in artificial light of intensity used was 12 hours, in buckwheat 17 hours.

LUBIMENKO and SZEGLOFF (18), in working with a range of shorter exposures (from 14 hours down to 4), found a greater depth of greenness in the longer durations, as did Miss DEATS in her 17.5-hour plants. General observations indicated lower chlorophyll content in the 5 and 7-hour plants than in the intermediate durations, with yellowing occurring to greater or less degree in the 19 and 24-hour exposures. Contrary to this last, BONNIER found greater amounts of chlorophyll in his continuous light plants. The majority of his plants were different from mine, it is not known that he used comparable intensity, and his experiments did not introduce the extra supply of carbon dioxide as a factor. These factors may account for differences.

Summary

1. In plants with short exposures to light, there are usually low carbohydrate and low protein reserves with less total growth and less production of differentiated tissues than in longer intervals.

2. In plants with longer light duration, there is increase in carbohydrate reserves without proportionately increased use in elaboration of proteins and tissue production.

3. Nitrates are present in great amounts in all tomato plants except the control and gantry crane house specimens. They are low in amount in buckwheat in 17, 19, and 24-hour exposures. They are usually lowest in the gantry crane house with extra carbon dioxide.

4. The maximum development of the plant (considering height and differentiated tissue) occurs in the 12-hour tomato and the 17-hour buckwheat.

5. Injury is evident in the tomato in exposures of 17 hours or more, resulting in marked decrease in photosynthetic ability.

6. Continuous light tends to produce thinner leaves, with palisade layer shorter or lacking.

7. The effect of conditions in the gantry crane houses as regards leaf thickness is variable.

8. Thinner leaves usually show an increase in the number of stomata, except in continuous light in intensity used.

9. Root development in fibrous systems appears roughly comparable with that of the aerial parts.

10. In the storage roots of four o'clock, maximum development occurs in the gantry crane houses and continuous light, while maximum height of stem occurs in the 17-hour plant.

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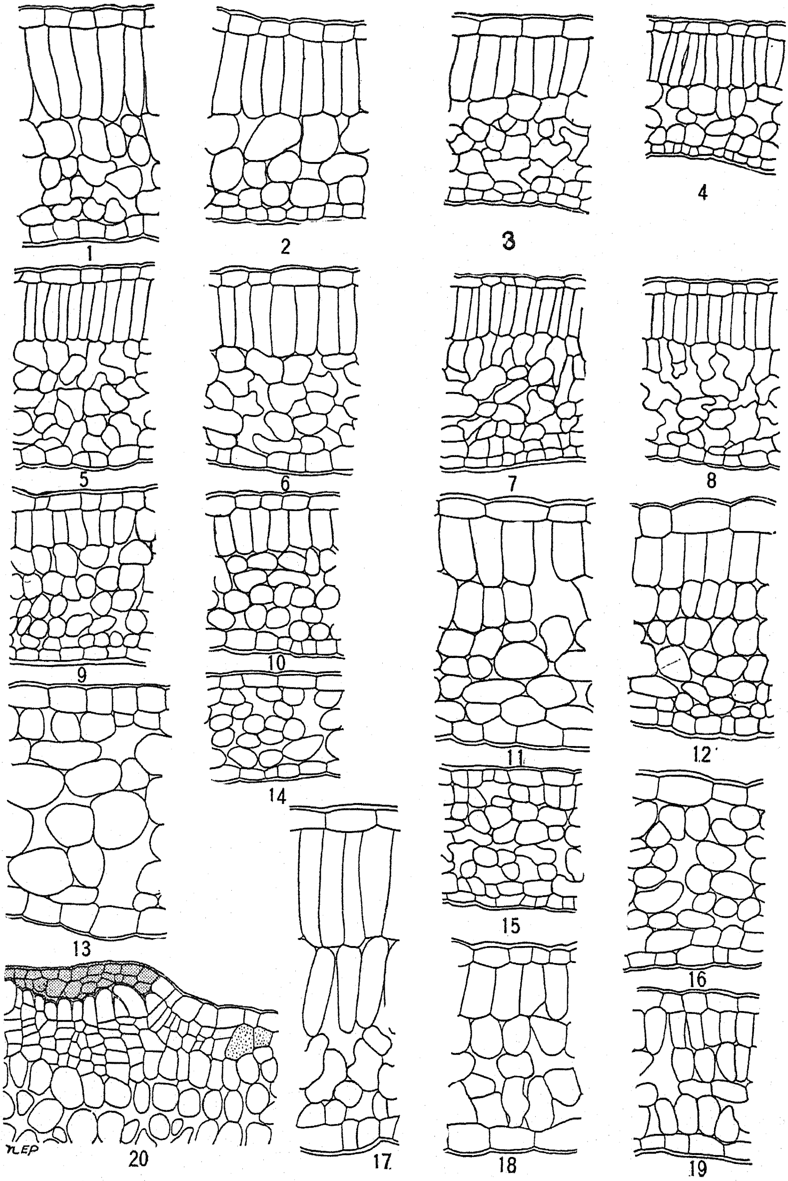

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EXPLANATION OF PLATE XV

FIGS. 1–4.—Pepper leaf: successive figures show structure in control, continuous light, and gantry crane house with extra carbon dioxide, and with usual atmosphere.

FIGS. 5–8.—Four o'clock leaf: successive figures as in pepper.

FIGS. 9–12.—Geranium leaf: successive figures as in pepper.

FIGS. 13–16.—Lettuce leaf: successive figures as in pepper.

FIGS. 17–19.—Soy bean var. Peking leaf: successive figures show control, gantry crane house with extra carbon dioxide, and with usual atmosphere.

FIG. 20.—Outer portion of tomato stem, showing injury in continuous light: shaded portion brown in living material; stippled cells are subepidermal ones which have retained green coloring; corklike cells between brown layer and collenchyma tissue.

GROWTH OF SEEDLING IN RELATION TO COMPOSITION OF SEED

MARY E. REID

(WITH PLATES XVI–XVIII)

Experiments with seedlings varying in their proportions of carbohydrates to nitrogen have been conducted by allowing the seedlings to grow on their own nitrogen reserves to maximum size in light, in darkness, and in light in atmospheres containing 0.4 per cent CO_2 and lacking CO_2,[1] except for small amounts given off in respiration. Corresponding tests have also been conducted in which the seedlings have been given nitrate nitrogen. The seedlings were grown in sterilized quartz sand, kept moist by the use of nutrient solutions. The object was to study the total growth-producing value of the foods stored in the different seeds, and the relations of varying proportions of these organic foods in altering the shoot to root ratios. Of particular interest have been the relations of total nitrogen to growth, and the modifying influences of additional carbohydrates, synthesized in the light, and of nitrate nitrogen on seedlings grown in light and in darkness. All records of growth are based on measurements of size and green weights. In calculating the efficiency of the food reserves for growth, the seed coats were removed.

1. Seedlings grown in darkness at 21°–24° C.

(*a*) ON THEIR OWN NITROGEN RESERVES.—The food reserves having a high proportion of nitrogen and fat have been the most efficient in producing growth in darkness. The total growth in grams of green material produced per gram of original dry food material ranged from 4.86 gm. for low-protein corn to 32.51 gm. for sunflower, a high-protein, high-oil seed. The shoot to root ratios ranged from 2.01 for low-protein wheat to 8.37 for sunflower. Similar differences between varieties occur as between species; for example, high-protein corn and high-protein wheat both have higher

[1] The measurements of CO_2 concentration were made by Dr. WARD B. DAVIS.

shoot to root ratios than the corresponding types that are low in protein.

(*b*) WITH NITRATES.—Only a few of the seedlings, and these to only a small extent, responded to nitrates with an increase in total growth. Nitrates increased the growth of high-protein seedlings in as many instances as in seedlings of low-protein seeds. In nearly all cases there has been some modification of the proportions of shoots to roots, the seedlings receiving nitrates having relatively the greater weight of shoots. It seems that, although nitrates in most cases do not increase the quantity of growth in darkness, they do have the capacity for modifying the type of response.

2. Seedlings grown in atmospheres containing different amounts of CO_2

The atmospheres used contained high concentrations (0.4 per cent) of CO_2 and no CO_2, except for small amounts given off in respiration, the experiment being conducted during the latter part of May and early in June. The average temperature was 25° C. Seedlings of each type were harvested when the culture of the particular type, which had the most limiting conditions for growth, had reached its maximum size. Cultures of each kind, grown with and without CO_2, were harvested on the same day.

(*a*) ON THEIR OWN NITROGEN RESERVES.—Seedlings from low-protein corn, when grown in an atmosphere lacking CO_2 except for small traces given off by the seedlings themselves in respiration, produced larger plants with greener leaves than did those which had 0.4 per cent CO_2. Seedlings from high-protein corn, however, grew more and had greener leaves in an atmosphere containing 0.4 per cent CO_2. Seedlings from other types of high-protein seeds, such as soy bean, cowpea, sunflower, and cantaloupe, all responded similarly to the high-protein corn, and were also greener when given CO_2 than when grown without it. Some kinds of seedlings had their total growth increased nearly 150 per cent by using CO_2. Even more striking than the effect of CO_2 on total growth, is its influence on the shoot and root ratios. Growth of roots was increased much more than growth of shoots in those seedlings whose total growth was increased by CO_2. Growth of roots of soy bean seedlings was increased

202 per cent more than that of the tops. From these results it seems that CO_2 must have had a modifying influence in the utilization of foods stored in the seed.

(*b*) WITH NITRATES.—Seedlings from low-protein seeds, such as low-protein corn, when grown on their own carbon supply, had their growth increased by the addition of nitrates. Seedlings from high-protein seeds, grown on their own carbon supply, varied in their behavior with respect to the addition of nitrates. Sunflower seedlings grew the same amount with nitrates as without, and muskmelon, cowpea, and soy bean seedlings grew somewhat more with nitrates. When grown in high concentrations (0.4 per cent) of CO_2, seedlings of both high and low-protein seeds showed increased growth with nitrates, but those of high-protein seeds had the greater increase. The response in total growth of seedlings of high-protein seeds to CO_2 in most cases was much greater than their response to nitrates. The response in total growth of seedlings of low-protein seeds to CO_2 was much less than their response to nitrates.

3. Seedlings grown in light in normal atmosphere

This experiment was conducted in October, the plants being grown in the greenhouse in daylight at an average temperature of 24° C. There were many cloudy days during the progress of the experiment. Each kind of seedling was allowed to grow until the seedlings of the lot grown without nitrates had reached their maximum size. The seedlings grown with and without nitrates for each type used were harvested at the same time.

(*a*) ON THEIR OWN NITROGEN RESERVES.—In most cases there was a greater total growth in light than there had been in darkness in the experiments previously described. Low-protein wheat, however, grew as much in darkness as in light; low-protein corn grew 45 per cent less in light than in darkness; and rice grew 43 per cent less in light than in darkness. Seedlings from high-protein seeds, on the contrary, grew much more in light than in darkness, and some of them produced blossom buds. Greater than the differences in total growth, however, were the differences in shoot to root ratios, relatively more roots being produced in light than in darkness. The greatest degree of shifting of ratios occurred in the seedlings of high-

protein seeds. With the exception of seedlings of the Gramineae, there were notable increases in leaf development in the light, as indicated by size, weight, and number of leaves.

(*b*) WITH NITRATES.—The seedlings may be classified into three groups with respect to their response to nitrates: seedlings of legumes, seedlings from low-protein and high-protein starchy seeds, and seedlings from high-protein, oily seeds. Seedlings of the leguminous type were the least responsive to nitrates. In general, they did not produce more than 30 per cent more total growth with nitrates than without, under the conditions of light and temperature used in this experiment. In previous experiments, conducted during the longer and sunnier days of May and early June, it had been shown that leguminous seedlings lacking nodules did have the ability to utilize nitrates. The tests with soy bean and cowpea seedlings, however, had indicated that increase of growth with nitrates did not occur to any extent except when photosynthesis of carbohydrates could go on at the same time. For this reason it is supposed that the failure to respond to nitrates during the shorter and somewhat cloudy days of October may be a seasonal condition, and may be connected with the lower light intensity and its resulting effects on photosynthesis of carbohydrates and proteins. Whatever may be the cause, whether difference in light intensity and duration, or temperature, or a combination of these factors, the fact remains that the legumes behaved in this experiment as a group that is characterized by a low grade ability to utilize nitrates.

Seedlings of the low-protein starchy seeds, all members of the grass family, in general produced the greatest response to nitrates in a given time. Low-protein corn gained 194 per cent more than high-protein corn as a result of using nitrates. Low-protein wheat gained 85 per cent more with nitrates than did high-protein wheat. Rice was injured by nitrates. The seedlings grew much larger without nitrates than with them. Seedlings of the high-protein, high-oil type responded more slowly to the influence of nitrates. During the first 10 days of growth, the seedlings with and without nitrates were not greatly different in size. Tomato and sunflower seedlings were exceptions; they showed well marked differences in the earlier stages of growth. It is supposed that the behavior of sunflower

seedlings is explainable because of their rapid response to CO_2 as discovered in a preceding experiment. It was then noted that sunflower seedlings responded remarkably to CO_2 even when nitrates were lacking, but that there was no response to nitrates when CO_2 was lacking. Tomato seedlings have not yet been tested in this way. At the time the plants were harvested, the seedlings of the high-protein, high-oil type presented notable differences when grown with and without nitrates. This was to some extent because they were allowed to grow for a longer time than seedlings of the low-protein, starchy type. In a future experiment it is planned to obtain the weights of the entire lot of seedlings at 5, 10, 15, and 20 day intervals. In this way it is hoped to present the quantitative and qualitative differences in the growth responses at different stages in the development of the seedlings of the various types studied.

With the exception of cotton seedlings, the shoot to root ratios of all types were increased by the addition of nitrates. In general, those seedlings whose total growth was most markedly influenced by nitrates were the ones whose shoot to root ratios showed the greatest increases. Seedlings of the Leguminosae were the least responsive in this respect, as they also were in their response in total growth.

The growth responses of the seedlings to varying proportions of carbohydrates to nitrogen agree with results obtained with tomato cuttings, described in former papers.[2]

Summary

1. Different kinds of seedlings produce very different growth responses when exposed to the same external influences. The responses are often related to the chemical composition of the seed.

2. If the nitrogen content of the seed is low in proportion to the carbohydrates, the response as measured by an increase in total growth in terms of green weight is greater when nitrates but no CO_2 (except traces given off in respiration) are utilized than when CO_2 but no nitrates are utilized.

[2] REID, MARY E., Relation of the kind of food reserves to regeneration in tomato plants. BOT. GAZ. 77:103–110. 1924.

———, Quantitative relations of carbohydrates to nitrogen in determining growth responses in tomato cuttings. BOT. GAZ. 77:404–418. 1924.

———, Growth of tomato cuttings in relation to stored carbohydrate and nitrogenous compounds. Amer. Jour. Bot. 13:1926 (detailed report).

3. If the nitrogen content of the seed is high in proportion to the carbohydrates, the response as measured by an increase in total growth is greater when CO_2 but no nitrates are utilized than when nitrates but no CO_2 (except for traces given off in respiration) are utilized.

4. The greatest total amount of growth, however, is produced by seedlings from both high and low-nitrogen seeds by allowing the seedlings to utilize both nitrates and CO_2.

5. The utilization of both nitrates and CO_2 tends to result in a shifting of the relative amounts of shoots and roots, that is, there are qualitative as well as quantitative responses to these external influences. The proportion of shoots to roots of low-protein seeds tends to be increased because of the relatively larger increase in the amount of shoots, if nitrates but no CO_2 is utilized; whereas the proportions of shoots to roots of seedlings of high-protein seeds are not increased and in some cases are even decreased by this treatment.

6. The shoot to root ratios of seedlings of low-protein seeds are not noticeably affected if CO_2 but no nitrates are utilized, but the shoot to root ratios of high-protein seeds tend to be greatly reduced if CO_2 but no nitrates are utilized. This shifting of ratios is a result of a greater increase in the amount of roots than of shoots.

7. The proportions of shoots to roots of seedlings of low-protein seeds tend to be slightly increased if both CO_2 and NO_3 are utilized, but the proportions of shoots to roots of seedlings of high-protein seeds are very greatly decreased by such treatment, because the weight and number of roots increase much more than the shoots.

8. The modification of type of growth is correlated with extent of modification in quantity of growth. Those treatments which result in the greatest increase in quantity of growth are the ones which produce the greatest change in type of growth.

(The foregoing statements apply to growth of seedlings in the light.)

9. A high-protein, high-oil food supply appears to be the most efficient in producing growth both in darkness and in light. This is not surprising, since both types of foods are constituted of a relatively large proportion of the condensed compounds of their respective types. For example, high-protein seeds contain relatively much

more of basic nitrogen and consequently have a great amount of nitrogen in proportion to their weight; oils contain a great amount of carbon in proportion to their weight.

10. The shoot to root ratios of seedlings grown in darkness appear to vary with the proportion of nitrogen to carbohydrates in the food reserves of the seed, the higher the nitrogen in proportion to carbohydrate content the higher the shoot to root ratios of the seedlings. There is some evidence that addition of nitrates to the culture medium of the seedlings grown in darkness can modify the type of growth (increase shoot to root ratios), but in only a few cases do nitrates increase the amount of growth.

11. Since wide variations in growth responses are associated with variations in the types and quantities of storage materials in the seed, it seems inadvisable to draw conclusions as to the effect of mineral nutrients or carbon dioxide treatment upon the subsequent growth of the plants when the observations are based on growth responses during the first three weeks of growth only. Before studying the effect of an element or compound on growth (in general), it would seem desirable to conduct experiments in which the storage supply of the element or compound in question has been at least partially exhausted, by allowing the seedlings to grow to maximum size without that substance. This method has been employed by animal workers for some time.

12. Although contradictory results have not been found in the different series of experiments, the results are not considered conclusive but will be repeated and elaborated.

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EXPLANATION OF PLATES XVI–XVIII

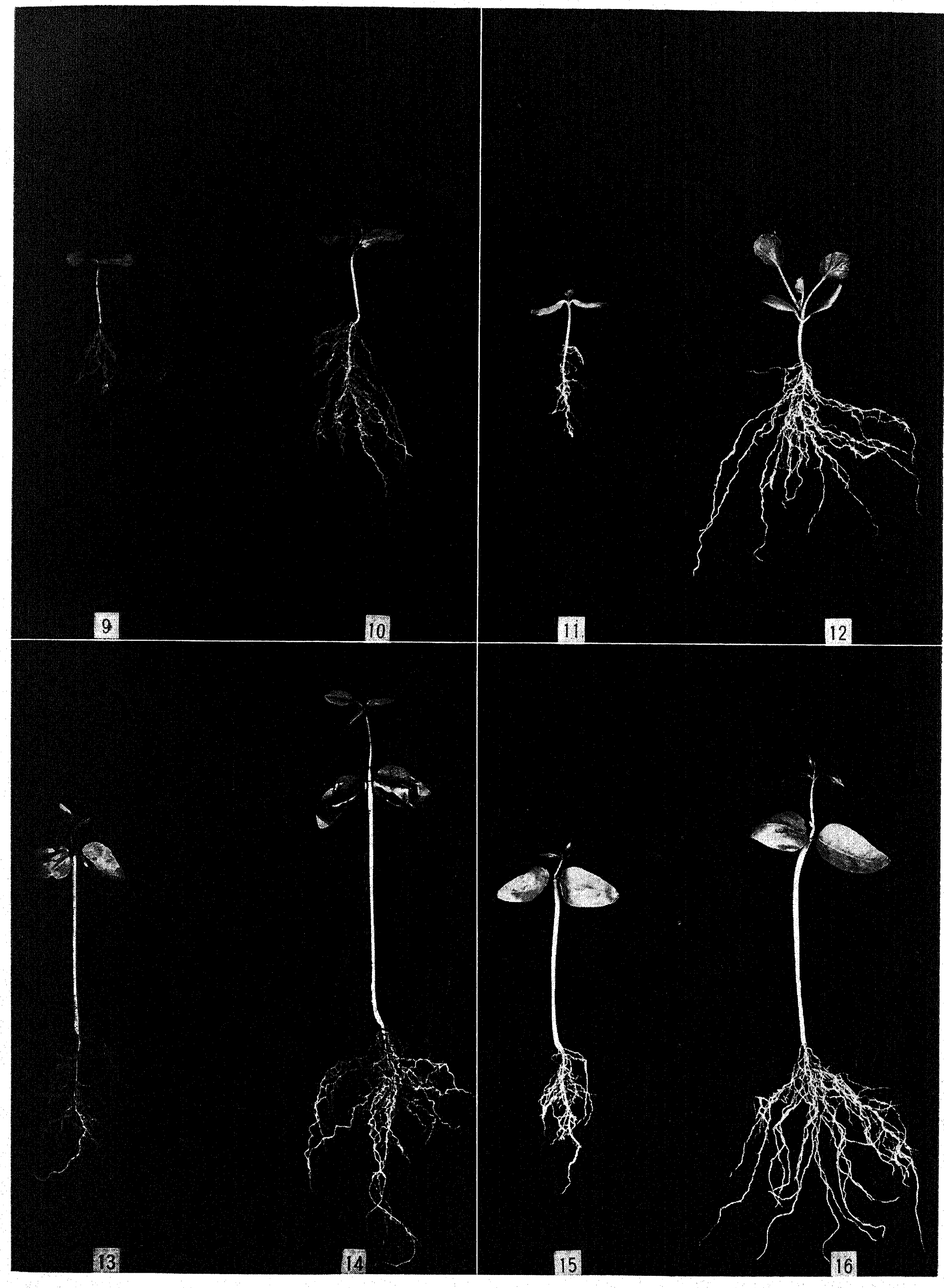
PLATE XVI

FIGS. 1, 2.—Illinois high-protein corn seedlings, grown without nitrates: former without CO_2, latter with CO_2.

FIGS. 3, 4.—Illinois high-protein corn seedlings grown with nitrates: former without CO_2, latter with CO_2.

FIGS. 5, 6.—Illinois low-protein corn seedlings grown without nitrates: former without CO_2, latter with CO_2.

REID on SEEDLING GROWTH



REID on SEEDLING GROWTH

REID on SEEDLING GROWTH

FIGS. 7, 8.—Illinois low-protein corn seedlings grown with nitrates: former without CO_2, latter with CO_2.

PLATE XVII

FIGS. 9, 10.—Rocky Ford melon seedlings grown without nitrates: former without CO_2, latter with CO_2.

FIGS. 11, 12.—Rocky Ford melon seedlings grown with nitrates: former without CO_2, latter with CO_2.

FIGS. 13, 14.—New Era cowpea seedlings grown without nitrates: former without CO_2, latter with CO_2.

FIGS. 15, 16.—New Era cowpea seedlings grown with nitrates: former without CO_2, latter with CO_2.

PLATE XVIII

FIGS. 17, 18.—Low-protein (Little Club) wheat seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

FIGS. 19, 20.—High-protein (Marquis) wheat seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

FIGS. 21, 22.—New Era cowpea seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

FIGS. 23, 24.—Hubbard squash seedlings grown in normal atmosphere: former without nitrates, latter with nitrates.

AN ORGANISM OF TOMATO MOSAIC

SOPHIA H. ECKERSON

(WITH PLATES XIX–XXII)

Introduction

This study was begun in 1922 at the University of Wisconsin, in connection with work on wheat rosette with H. H. McKINNEY, while on part-time appointment from the Cereals Division of the United States Department of Agriculture. The early studies of tomato mosaic were made in 1922 and 1923, with the cooperation of E. J. KRAUS of the University of Wisconsin. Dr. KRAUS also turned over to me abundant material, both diseased and healthy plants, for the study of mosaic in *Hippeastrum Johnsoni* and pepper. The figures in pl. XIX were drawn from slides made at that time. The work has been continued at Boyce Thompson Institute. At present H. R. KRAYBILL and the writer are starting a study combining differential filtration, inoculation, and microchemical examination, which should bring out new facts about mosaic. In the meantime, I shall describe organisms seen in mosaic plants, especially the one in tomato, which has been observed most carefully.

Motile organisms

WHEAT.—Motile organisms were first seen in the rosette wheat. In the first few days of the infection there were numerous tiny flagellated forms (2–4 μ) in the cells. A week later there were fewer of these, but many larger motile forms (5–7 μ). Still later the large non-motile bodies predominated. These different forms always appeared in this sequence.

TOMATO.—An examination of mottled leaves of mosaic plants available revealed motile organisms in all. The very young, not yet mottled leaves of the mosaic plants also had hundreds of tiny rapidly moving organisms (fig. 5 right) in the mesophyll cells; while in the phloem cells were elongated forms having typical flagellate movement. In the older, badly mottled leaves were many melon-seed-

shaped, sporelike forms having a hyaline membrane (fig. 8 right). These were imbedded in the few remaining chloroplasts as well as free in the cells. These sporelike forms were puzzling, since they were not stainable, either living or in smears. More is known about them now, but they are still somewhat puzzling.

VITAL STAINS.—Much time was given to observation of the living organisms within the living cells of the plant. Of the many vital stains tested for differentiation, the combination of methylene blue and eosin was the most satisfactory for the small forms. These small ovoid forms, having amoeboid movement (fig. 6), and the long thin flagellate forms stain readily. When the stains are used in very dilute solution, the organisms are tinged with the eosin, while their nuclei are blue. Recently I have used polychrome methylene blue instead of the eosin combination. Since the nuclei of these small organisms are denser than the somewhat transparent body, they can be seen clearly in living unstained condition by proper arrangement of the light.

Brilliant cresyl blue is satisfactory for the larger forms (fig. 7 left). If used in combination with acid fuchsin, the organisms are tinged with red, while their nuclei are blue. This combination was also used with good results on wheat rosette for distinguishing the big bodies from the cell nuclei. In that case the stains were made up in formalin.[1] The bodies were pink with blue nuclei. The cell nuclei were blue with pink nucleoles. This was excellent for diagnosis, but the colors were not permanent.

Sections of fresh tissue were stained also with iron haematoxylin and with azur-eosin under observation at the microscope. This was for localization of the organisms, and to be certain that the ones stained in the smears were those observed living in the cells.

ORGANISMS IN CHLOROPLASTS.—This year Dr. KRAYBILL and I have inoculated several series of young tomato plants with filtered juice from mosaic plants. Alternate leaflets of three or four leaves on each plant were scratched with a fine needle, and the juice brushed over them. I examined the uninoculated leaflets, opposite

[1] (1) 0.25 gm. brilliant cresyl blue, 90 cc. 4 per cent formalin, and 10 cc. methyl alcohol; (2) 0.05 gm. acid fuchsin, 0.05 gm. orange G, 95 cc. 4 per cent formalin, and 5 cc. 1 per cent acetic acid. Mix a few drops of (1) and (2); stain lightly (about 10 min.).—Enzlyk Mik. Tech. 1910.

those inoculated, at intervals of twenty-four hours for the first few days.

Twenty-four hours after inoculation, in leaflets opposite inoculated leaflets, there were tiny flagellated organisms (fig. 13 and fig. 15 extreme left) in the veins and in the adjacent mesophyll cells. None were found in other regions. Most of the chloroplasts of these cells were in healthy condition. Many had several small starch grains at the surface (fig. 9 left), but in a few of the cells nearest the veins the chloroplasts were beginning to show signs of dissolution. Small organisms appeared to be entering some of these plastids (fig. 9 middle), while they had entered and were beginning to swim around in little pools in others (fig. 9 right).

Three days after inoculation there were tiny flagellated forms, and also somewhat larger forms (fig. 14) in great numbers throughout the mesophyll tissue.

Five days after inoculation many cells of the mesophyll tissue were in bad condition. Their chloroplasts were no longer held by the cytoplasm in orderly arrangement, but were floating free. Most of these chloroplasts showed progressive liquefaction, while the organisms within them were larger (fig. 10 left and middle). Many of the chloroplasts were almost wholly liquefied (fig. 10 right and fig. 11 left).

Seven days after inoculation some chloroplasts of the palisade cells were in process of liquefaction. Many of the remaining plastids contained non-motile bodies which seemed to be early stages of spore formation (fig. 11 middle and right).

Ten days after inoculation some of the leaflets were beginning to show mottling. Within the leaf groups of palisade cells were partially disorganized; the cytoplasm was gone (or liquefied); and the chloroplasts were in disarray, many partially dissolved, others containing spores (fig. 12). These groups of disorganized cells were usually bounded by groups of cells apparently in perfectly healthy condition.

The foregoing description is from our latest series. The specific number of days after inoculation for any stage varied a little in the different series, but the sequence was the same in all. Moreover, the sequence in inoculated plants is the same as that from the youngest

leaf of any mosaic plant down to the fifth or sixth from the tip. The only difference found was in the later stages of the disease. In plants growing under good cultural conditions in the field, there are fewer spores but many more big slowly motile forms (fig. 16).

SPORES.—Twenty to thirty days after inoculation, although there were still groups of good cells, most of them had become filled with melon-seed-shaped spores. These cells were completely disorganized, without either cytoplasm or chloroplasts. They were like little boxes filled with pebbles which spilled out when the walls were cut.

The mature spores (fig. 8 right) have a highly refractive, hyaline membrane of slight permeability, and are difficult to stain. Recently, however, rather good results have been obtained by fixing in methyl alcohol, staining overnight in iron haematoxylin, and destaining.

Before the spores are mature, that is before the hyaline membrane is formed, the walls are very permeable. Their contents are difficult to stain in place, because they are shot out during fixation. Many spore cases show a spiral filament still attached. There are always dozens of detached tails on the slide, and among these many tiny flagellated forms. On one particularly good slide the tiny organisms were in such a position on the filament that it seemed that they must have been shot out together (fig. 18).

Peculiar bodies, appearing much like Japanese lanterns in the living tissue, were caught in two of the inoculated series (fig. 17 left two figs.; fig. 18 left). When stained with haematoxylin they show clearly two or three organisms within each. These tiny organisms are the same type as those present at the beginning of the disease (fig. 13 left).

Organisms in other mosaic plants

For comparison with tomato mosaic, several other plants were examined. I found similar organisms in all, although not necessarily the same organism. There are slight differences in form and considerable differences in size, but the general appearance and behavior are the same. There are tiny organisms in the youngest leaves, also in inoculated plants of *Hippeastrum Johnsoni* (fig. 2). There are elongated flagellate forms in the phloem tissue of older leaves (*Hip-*

peastrum Johnsoni, fig. 3; *Dahlia*, fig. 22 left; squash, fig. 25 left). There are also the larger slow moving forms (*H. Johnsoni*, fig. 4 left; squash, fig. 26 left). In badly mottled leaves of all there are spore forms (*Dahlia*, fig. 23 right; squash, fig. 26 right; *H. Johnsoni*, fig. 4 right).

The spores from *Dahlia* should be good material for a study of development, structure, contents, and germination of these, as yet little understood bodies. I hope to make such a study next summer.

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EXPLANATION OF PLATES XIX-XXII

All figures were drawn with a Bausch & Lomb 1.8 mm. fluorite objective, 1.3 N. A., and Leitz periplane ocular 20X. All magnifications are approximately 3200 diameters.

PLATE XIX

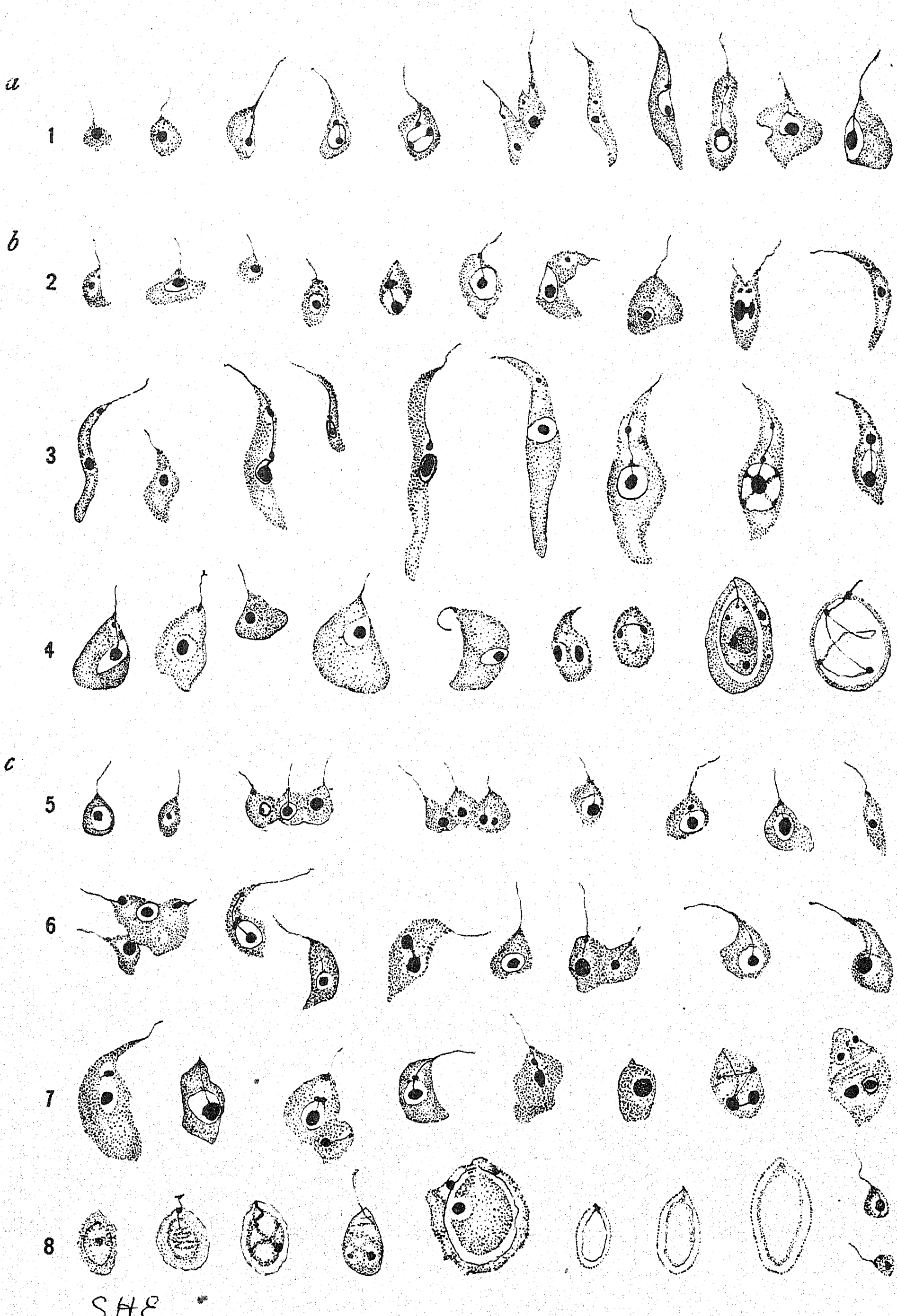
FIG. 1.—Pepper, with iron-alum haematoxylin: tiny flagellated forms; elongated forms; at right, amoeboid forms.

FIGS. 2–4.—*Hippeastrum Johnsoni*, with iron-alum haematoxylin: fig. 2, from young leaf of seedling 10 days after inoculation; fig. 3, elongated flagellate forms from phloem of leaf of mature plant; fig. 4, from badly mottled leaf; at right, stages in spore formation.

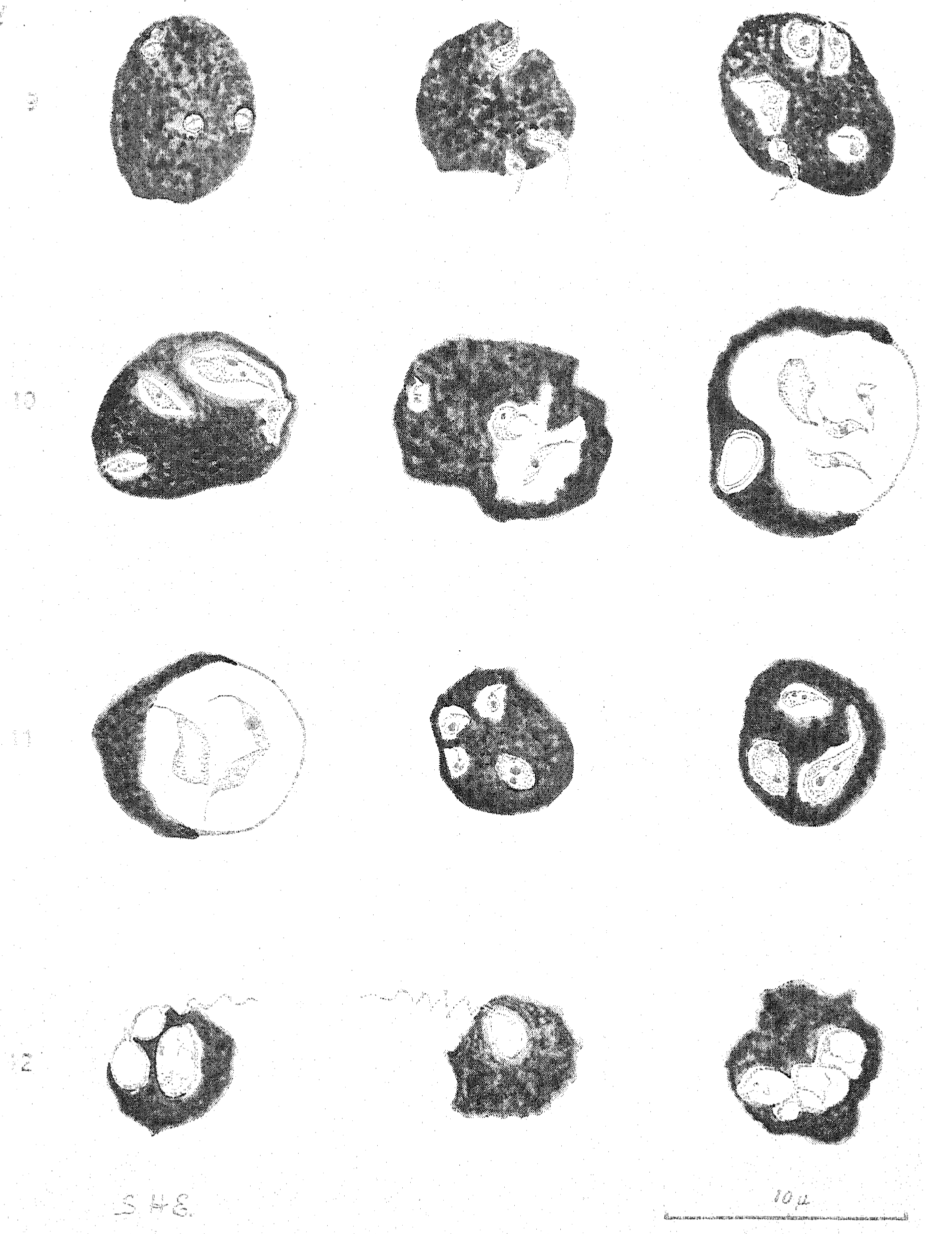
FIGS. 5–8.—Tomato, with azur-eosin: fig. 5, from youngest leaf of mosaic plant, division; fig. 6, from young mottled leaf; fig. 7, three figs. at right probably stages in spore formation; fig. 8, stages in spore formation; at right three spores with hyaline membranes; a few tiny flagellated forms found on slide among these spores.

PLATE XX

FIGS. 9–12.—Tomato, chloroplasts from leaflets opposite inoculated leaflets; with two exceptions fixed in 1 per cent osmic, stained with iron haematoxylin 10 minutes at 45° C.: fig. 9, from mesophyll cells bordering veins, 24 hours after inoculation; left figure with small starch grains, which show clear cross in polarized light; middle figure, tiny organisms entering plastid; right figure, organisms in plastid, which is beginning to liquefy; fig. 10, from mesophyll cells 5 days after inoculation; increasing liquefaction of plastid; right figure from living unstained tissue; nuclei seen by oblique light; nucleus not visible in the denser organism; spore with hyaline membrane imbedded in remainder of plastid; fig. 11, left figure similar to right figure of fig. 10; middle and right figures from palisade cells 7 days after inoculation; fig. 12, from palisade cells 10 days after inoculation; leaflet slightly mottled; spiral filaments shot out by osmic acid.



ECKERSON on TOMATO MOSAIC



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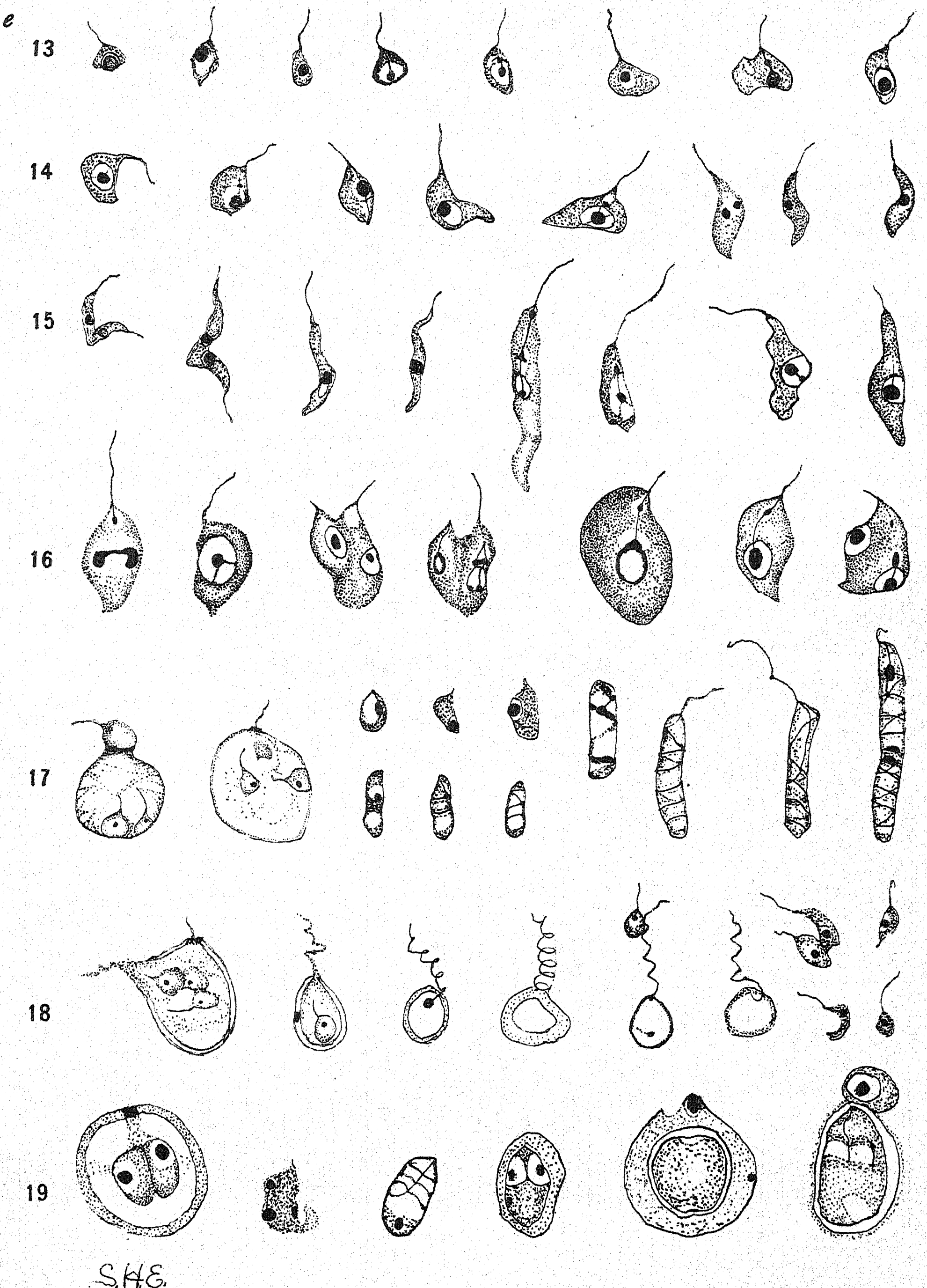
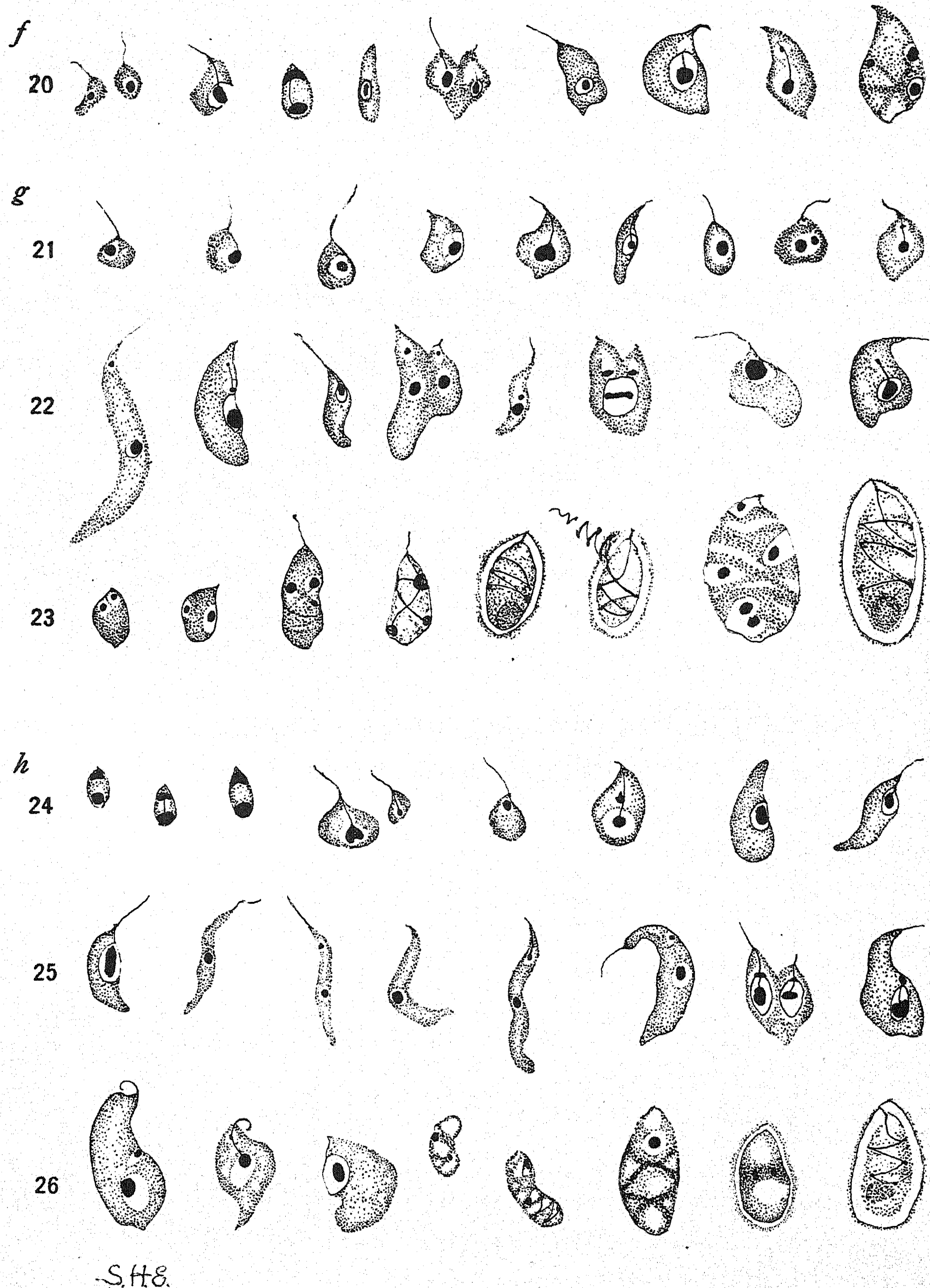


ECKERSON on TOMATO MOSAIC



ECKERSON on TOMATO MOSAIC

PLATE XXI

FIGS. 13–19.—Tomato: fig. 13, tiny flagellated forms having rapid buzzing movement, predominating in youngest leaf, also in leaf the first few days after inoculation; fig. 14, larger forms having active amoeboid movement, found in second and third leaf below tip, also in leaf third and fourth days after inoculation; fig. 15, elongated forms having typical flagellate movement, found chiefly in phloem parenchyma; fig. 16, larger less active (except when dividing) forms from fifth and sixth (from tip) mottled leaves from large plants in garden; fig. 17, two figures at left from palisade cells 5 days after inoculation; figures at right probably spore forms, from phloem cells; fig. 18, two figures at left containing tiny organisms similar to those in fig. 13; spores having spiral filaments, extruded during fixation; at extreme right tiny flagellate forms seemingly ready to start the cycle anew; fig. 19, stages in formation of large spores, found chiefly in badly mottled leaves from garden.

PLATE XXII

FIG. 20.—Strawflower: figures at leaf from youngest leaf; at right from mottled leaf; figure at extreme right beginning spore formation (?).

FIGS. 21–23.—Dahlia: fig. 21, from yellow tip of stunted badly diseased plant; fig. 22, from older mottled leaf; fig. 23, from large mottled leaf of vigorous shoot, stages in spore formation.

FIGS. 24–26.—Squash: fig. 24, from young leaf of badly diseased and distorted plant; fig. 25, elongated flagellate forms; fig. 26, from badly mottled leaf; at left large forms having slow movement; at right spore formation.

ATTEMPT TO CULTIVATE AN ORGANISM FROM TOMATO MOSAIC[1]

HELEN A. PURDY

Introduction

In discussing the etiology of diseases of man and animal produced by filterable viruses, the consensus of opinion is in favor of the theory that they are caused either by microorganisms not demonstrable by our present methods, or by ultramicroscopic organisms (9, 10, 13, 18). This conclusion is based upon the fact that the active agent in the filtrate apparently multiplies within the living host, since an infection obtained by inoculation with the filtrate can be transferred successively through a series of animals after the dilution of the original inoculum has exceeded the point capable of reproducing the disease. Although the virus of tobacco mosaic exhibits the same property of indefinite multiplication within living plants, not all plant pathologists regard this evidence as sufficient support for the parasitic theory of mosaic diseases.

Many hypotheses have been proposed to explain the origin of tobacco mosaic as a purely physiological response to unfavorable environmental conditions of the soil or climate (3, 5, 7, 14, 15), or due to various agents such as a "contagium vivum fluidum" (1), unorganized ferments or toxins (6, 7), oxidizing enzymes (2, 4, 16, 17), and only recently to potato protoplasm (8). It seems likely that all of these hypotheses would be superseded by the parasitic theory, if the virus could be multiplied *in vitro*.

Many unsuccessful attempts have been made to cultivate a causative organism from plants affected with mosaic disease. Considerable interest, therefore, was aroused when OLITSKY (12) recently reported success in cultivating *in vitro* an active agent that would produce mosaic disease in tobacco and tomato plants. The importance of OLITSKY's conclusions makes a careful repetition of his experiments desirable. The investigations here reported were

[1] The writer is indebted to Dr. L. O. KUNKEL for valuable suggestions and a critical review of the manuscript.

undertaken with the purpose of repeating his recent work. With a few modifications, the methods employed by OLITSKY were carefully duplicated. While this work was in progress, MULVANIA (11) reported that he had repeated OLITSKY'S work, but was unable to obtain any evidence that the active agent of mosaic disease multiplies outside the living tobacco and tomato plants.

The stock virus used was extracted from mosaic-affected tomato plants in a greenhouse of the Boyce Thompson Institute. Although OLITSKY obtained his tomato mosaic virus from the same source a year previously, the writer has no proof that the two stock viruses were identical.

Method

PREPARATION OF MEDIUM.—Eighty gm. of tomato shoots, 5–6 weeks old, were minced with scissors and ground to a soft pulp in a sterile mortar; 250 cc. of distilled water added, and the entire mixture centrifuged at high speed for one hour. The supernatant fluid was passed, first through a paper pulp suction filter to remove the bulk of plant tissue, then through a sterile Berkefeld "W" under strictly aseptic conditions, and finally through a second similar Berkefeld filter.[2] The hydrogen-ion concentration of the filtrate was determined by the electrometric method. The reaction of the various lots of medium gave a range of P_H 5.08–6.26.[3] The medium was tubed and incubated at 28°–30° C. for 7 days. At the end of this period of incubation, all tubes showing evidence of contamination or a precipitation of albumins and globulins were discarded. Only the tubes that contained clear media were used.

TESTING OF MEDIUM.—At the end of a 7-day period of incubation, a tube of medium was inoculated into 16 healthy tomato plants. After a second week of incubation, the inoculation of 16 additional healthy plants was repeated. If the 32 plants inoculated with a given lot of medium remained healthy, the medium was considered free from virus.[4]

CULTURE.—The stem of a badly affected mosaic tomato plant

[2] OLITSKY used a Berkefeld "N" and no paper pulp suction filter.

[3] The media used by OLITSKY gave a reaction of P_H 5.3–6.0.

[4] Considerable difficulty was experienced in obtaining medium free from virus.

was cut with a razor. The cut end was flamed, and a sterile capillary pipette was inserted in the stem, from which approximately 0.01 cc. of juice was withdrawn and inoculated into 5 cc. of medium. The culture was then incubated at 28°–30° C. After 7–14 days, subplants were made by adding 0.5 cc. of the original culture, thoroughly rolled to insure mixing, to 5 cc. of medium. Subsequent subplants were made in a similar manner, and the approximate dilution of the introduced virus was estimated.

INOCULATION.—Vigorous tomato plants about 6 weeks old were inoculated by scarifying one leaf on each of three separate branches of the same plant and rubbing in the inoculum with a cork. Cognizant of the fact that a plant, apparently healthy, might already be affected with mosaic disease in its incipient stages, every precaution was taken throughout inoculation to safeguard against a chance transmission of mosaic from one plant to another. The leaves to be inoculated were held by means of a separate paper slip for each plant; the needle used for scarifying the leaves was sterilized by flaming after each inoculation; and an individual sterile cork was employed for rubbing in the inoculum. Moreover, all inoculated plants, including those used for control, were held together for four weeks in the same greenhouse, which was kept carefully fumigated.[5] Under these conditions, all of the plants were exposed equally to the risk of accidental infection.

CONTROLS.—In every experiment, in addition to the medium inoculated with virus, two tubes, each containing 5 cc. of medium from the same lot, were included. One was uninoculated; the other inoculated with 0.01 cc. of juice from a healthy tomato plant. These control tubes were treated in every way like the virus cultures. Subplants were made, using the same lot of medium to which the corresponding virus culture had been transplanted. A tube containing 5 cc. of sterile tap water inoculated with 0.01 cc. of juice from a mosaic affected tomato plant was also included in every experiment.[6] Subplants of the water culture corresponding to those of the virus cultures were made in sterile tap water.

[5] Thanks are due FREDERICK E. HEINSOHN for cooperation in keeping the greenhouse free from insect pests.

[6] MULVANIA introduced this control in his repetition of OLITSKY'S experiments.

Results

Of the plants that were inoculated from virus cultures containing original inoculum in an estimated dilution of approximately 2×10^{-3}, 41 of 50, or 82 per cent developed mosaic disease (table I). Subplants from these cultures produced infection in 13 of 70, or 19 per cent of the plants receiving virus in a dilution amounting to 2×10^{-4}. Upon reaching the dilution 2×10^{-5}, the virus cultures infected 9 per cent, or 6 of the 70 plants inoculated. At the next subplant, a 2×10^{-6} dilution was obtained that proved incapable of producing mosaic in any of the 70 plants into which it was introduced.

Of the five separate virus cultures made, three lost their power of infectivity upon reaching a dilution of the original 0.01 cc. of virus approximating 2×10^{-5} or 1–50,000, while the remaining two produced no mosaic at the next subplant, which was approximately a 2×10^{-6} or a 1–500,000 dilution.

The two water cultures lost their power of infectivity at a dilution of the original 0.01 cc. of virus approximating 2×10^{-5} or 1–50,000[7] (table II).

A total of 284 plants used in the control experiments remained healthy. Some were inoculated with plain medium; others with medium inoculated with juice from a healthy tomato plant.

Discussion

In the tabulated results it will be noted that the power of infectivity of each original virus or water culture and of every subplant in each series was tested and recorded. By this method, any appreciable multiplication of the active agent of mosaic disease may readily be detected. Also, the power of infectivity of the virus cultures can unquestionably be attributed to the 0.01 cc. of virus introduced in the original cultures, since the results of the control experiments prove that all the culture media used were free from any trace of virus. Also, by testing the entire series of subplants of a given culture successively, accidental infection from other sources than contaminated medium can more easily be detected, since a culture producing infection at the fourth subplant, when the third was non-

[7] MULVANIA reported a higher percentage infection from his water cultures than from his virus cultures at a given dilution.

TABLE I

POWER OF INFECTIVITY OF VIRUS CULTURES AT VARIOUS DILUTIONS

Culture	Period of incubation (days)	P_H of medium	Approximate dilution of original virus	No. of plants inoculated	No. of plants diseased*	No. of plants healthy*	Per cent infection	Average per cent infection
A...	14	6.19	2×10^{-3}	10	10	0	100	
B...	14	6.19	2×10^{-3}	10†	2	2	50	
C...	7	5.69	2×10^{-3}	2	2	0	100	
D...	7	5.69	2×10^{-3}	2	1	1	50	82
E...	7	5.69	2×10^{-3}	2	2	0	100	
C...	14	5.69	2×10^{-3}	10	10	0	100	
D...	14	5.69	2×10^{-3}	10	6	4	60	
E...	14	5.69	2×10^{-3}	10	8	2	80	
A^1‡.	7	6.19	2×10^{-4}	10	5	5	50	
B^1..	7	6.19	2×10^{-4}	10	0	10	0	
A^1..	14	6.19	2×10^{-4}	10	4	6	40	
B^1..	14	6.19	2×10^{-4}	10	0	10	0	19
C^1..	14	6.19	2×10^{-4}	10	0	10	0	
D^1..	14	6.19	2×10^{-4}	10	2	8	20	
E^1..	14	6.19	2×10^{-4}	10	2	8	20	
A^2§.	14	5.69	2×10^{-5}	10	0	10	0	
B^2..	14	5.69	2×10^{-5}	10	0	10	0	
$1A^2$..	14	5.69	2×10^{-5}	10	4	6	40	
$1B^2$..	14	5.69	2×10^{-5}	10	0	10	0	9
C^2..	14	6.19	2×10^{-5}	10	2‖	8	20	
D^2..	14	6.19	2×10^{-5}	10	0	10	0	
E^2..	14	6.19	2×10^{-5}	10	0	10	0	
A^3..	14	5.69	2×10^{-6}	10	0	10	0	
B^3..	14	5.69	2×10^{-6}	10	0	10	0	
$1A^3$..	14	5.08	2×10^{-6}	10	0	10	0	
$1B^3$..	14	5.08	2×10^{-6}	10	0	10	0	0
C^3..	14	6.10	2×10^{-6}	10	0	10	0	
D^3..	14	6.10	2×10^{-6}	10	0	10	0	
E^3..	14	6.10	2×10^{-6}	10	0	10	0	
$1A^4$..	14	6.26	2×10^{-7}	10	2¶	8	20	
$1B^4$..	14	6.26	2×10^{-7}	10	0	10	0	
C^4..	14	5.90	2×10^{-7}	10	0	10	0	4
D^4..	14	5.90	2×10^{-7}	10	0	10	0	
E^4..	14	5.90	2×10^{-7}	10	0	10	0	
$1A^5$..	14	5.90	2×10^{-8}	10	0	10	0	
$1B^5$..	14	5.90	2×10^{-8}	10	0	10	0	
C^5..	14	5.90	2×10^{-8}	10	0	10	0	0
D^5..	14	5.90	2×10^{-8}	10	0	10	0	
E^5..	14	5.90	2×10^{-8}	10	0	10	0	
			Total	366				

* Final number recorded four weeks after inoculation.

† Six inoculated plants were discarded accidentally a few days after inoculation.

‡ A^1 is the first subplant of A, A^2 is a subplant of A^1, etc.

§ A^2 and $1A^2$ are both subplants of A^1. A^2 was made when A^1 had been incubated one week, $1A^2$ after A^1 had been incubated two weeks.

‖ Accidental?

¶ These plants were standing beside plants affected with mosaic; doubtless accidental infection since the culture was non-infectious at the preceding dilution.

infectious, would be a questionable result, requiring substantiation before the infection could definitely be attributed to virus present in the culture (table I, footnotes ||, ¶; table II, footnote §).

The fact that the two water cultures did not exhibit the high

TABLE II

POWER OF INFECTIVITY OF WATER CULTURES AT VARIOUS DILUTIONS

Culture	Period of incubation (days)	Approximate dilution of original virus	No. of plants inoculated	No. of plants diseased*	No. of plants healthy*	Per cent infection	Average per cent infection
X	14	2×10^{-3}	10	2	8	20	9
Y	7	2×10^{-3}	2	0	2	0	
Y†	14	2×10^{-3}	10	0	10	0	
X[1]‡	7	2×10^{-4}	10	0	10	0	6
X[1]†	14	2×10^{-4}	10	0	8	0	
Y[1]	14	2×10^{-4}	10	2§	10	20	
X[2]||	14	2×10^{-5}	10	0	10	0	0
1X[2]¶	14	2×10^{-5}	10	0	10	0	
Y[2]	14	2×10^{-5}	10	0	10	0	
X[3]	14	2×10^{-6}	10	0	10	0	0
1X[3]	14	2×10^{-6}	10	0	10	0	
Y[3]	14	2×10^{-6}	10	0	10	0	
1X[4]	14	2×10^{-7}	10	0	10	0	0
Y[4]	14	2×10^{-7}	10	0	10	0	
1X[5]	14	2×10^{-8}	10	0	10	0	0
Y[5]	14	2×10^{-8}	10	0	10	0	
		Total	152				

* Final number recorded four weeks after inoculation.

† This culture is a portion of the preceding one which has been incubated one week longer.

‡ X[1] is the first subplant of X, X[2] is a subplant of X[1], etc.

§ Accidental?

|| Subplant of X[1] after incubating X[1] for one week.

¶ Subplant of X[1] after incubating X[1] for two weeks.

power of infectivity that the virus cultures of corresponding dilutions showed, cannot be explained adequately by assuming on the one hand that the tap water had an inhibiting or perhaps deleterious effect upon the active agent of the fresh virus, or by claiming on the other hand a slight multiplication of the virus in the medium used in the virus cultures. The number of water and virus cultures tested is too small, and the method of introducing the original inoculum does not permit of enough accuracy for fair comparisons. More significant

conclusions can be drawn by comparing the percentage infection exhibited by successive subplants of the same series.

Conclusion

In the experiments here reported, in which the methods of OLITSKY were followed, the writer has been unable to obtain any evidence that the active agent producing mosaic disease in tobacco and tomato plants multiplies outside the living plants.

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TWO NEW SPECIES OF ZAMIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 345

CHARLES J. CHAMBERLAIN

(WITH SIX FIGURES)

Genera in the cycads are not only so distinct that they are easily recognizable, but they are so sharply defined that interrelationships within the family are uncertain. Sister MARY ALICE[1] constructed a key to the genera, based upon the leaflets. A similar key, impractical to apply, but nevertheless effective wherever material is available, could be based upon pollen tube structures. The usual keys, based upon cones, are very effective when cones are available.

Some species are almost as sharply limited as the genera; but others, especially in the larger genera, present such variation that identification is difficult, and it is likely that new species have been described when no new description was needed. In *Macrozamia* there is a plexus of forms, with *M. spiralis* as a center, which might afford a better study in variation than in taxonomy, for the description of species might degenerate into a description of individuals. There are similar centers in all the larger genera, especially in *Zamia*, which contains more than a third of all the species in the family. Consequently, one should exercise some caution in describing new species in cycads. Nevertheless, there are doubtless many new species of this family still waiting to be discovered and described. Two species of *Zamia*, from Mexico, are sufficiently distinct to be described as new. One I raised from a seed secured near Jalapa, and the other I dug up near Tuxtepec. The former has been under observation in my collection for nearly twenty years, and the latter for fifteen years.

Before venturing to describe these plants as new species, I examined all the *Zamia* material at Kew, both in the herbarium and in the greenhouses. I also had the pleasure of studying the collection in the greenhouse and herbarium of the New York Botanical Garden, doubtless the most extensive collection of *Zamia* in the world. Dr.

[1] LAMB, SISTER MARY ALICE, Leaflets of Cycadaceae. BOT. GAZ. 76:185–202. 1923.

N. L. BRITTON, Director of the Garden, went through the entire collection with me, giving the benefit of his extensive acquaintance with this genus in the field, and also of his long experience in taxonomy. Not being a taxonomist, I might have hesitated to describe the species without this assistance; but a description seemed necessary, since the species from the Jalapa neighborhood has been used in hybridizing, and is also being used in a cytological study of the determination of sex.

Zamia monticola

This species was grown from a seed collected on the steep mountain side about six or eight miles west of Jalapa, opposite the extinct crater of Naolinco. The mountain side is densely covered with shrubs and small trees, with a rich herbaceous undergrowth. A *Begonia* was found here with a leaf which measured a meter across. This is the best region for *Ceratozamia* encountered during several collecting trips in the Mexican tropics. *Ceratozamia* is very abundant on the steep slopes and it cones freely. In 1906, I picked up a great number of seeds of *C. mexicana*. Dr. C. R. BARNES and Dr. W. J. G. LAND were with me on that trip, and none of us noticed any *Zamia* in this region. When the seeds were planted, all the pots were marked *C. mexicana*, and not until the seedlings were well developed was *Ceratozamia* no. 10 noticed to be a typical *Zamia*.

On August 8, 1915, nearly eleven years from the planting of the seed, a male cone appeared, followed at intervals of about a week by three others. These were typical *Zamia* cones, appearing in succession, so that when the first cone was 8 cm. in length, exclusive of the peduncle, the others measured respectively 6, 4, and 3 cm. At the shedding stage, the first cone had reached a length of 16 cm., and its peduncle measured 17 cm. The other three cones were slightly smaller when they reached the shedding stage.

Since 1915 this plant has coned five times, producing four cones in 1918, six in 1920, six in 1922, two in 1923, five in 1924, and four in 1925. The cones are all large for the genus, the first cone in each set reaching a length of 12–16 cm. at the shedding stage. The peduncles, which are as long as the cones, are seldom erect; more often they are nearly horizontal and curved (fig. 1). Of the six cones produced in 1920, the first began to shed its pollen on December 14,

and the last on February 9, 1921; so that the period of shedding pollen, while not continuous, extended over about two months, a very favorable feature in securing hybrids.

The sporangia are in two rather widely separated groups, with 10–16 sporangia in a group. Nearly all the sporangia are in pairs,

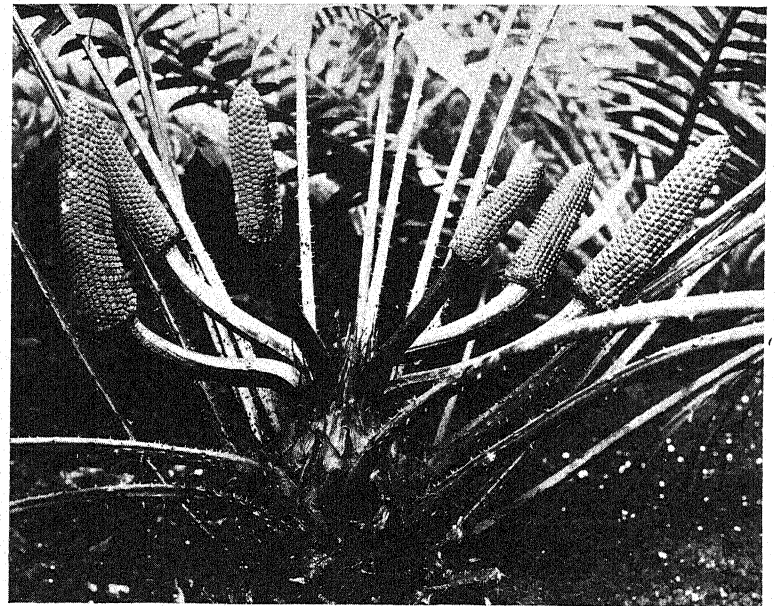

FIG. 1.—*Zamia monticola*, cones of 1920: cone at left reached length of 16 cm. when pollen was shed; leaves in background belong to *Encephalartos;* negative taken in University of Chicago greenhouse by P. J. SEDGWICK.

so that there are only two sporangia in a sorus. Some of the sporangia are single, and, in a few cases, sori of three sporangia were noticed. Nearly all of the sporangia are in rows, with the dehiscence in the longitudinal axis of the sporophyll. All of these features are shown in fig. 2.

Ever since the plant began to cone, new leaves have been produced with each set of cones. In 1919 the crown consisted of six leaves, which reached a length of 1.7 m. The crown of 1923 contained eighteen leaves, and the latest crown, that of 1925, consists of twenty-one leaves, the largest of which are 1.7 m. in length.

The lower part of the petiole, below the lowest leaflets, is very spiny, but there are very few spines in the leafy portion. The number of leaflets varies from thirty to thirty-four, the upper twenty of which are approximately in pairs; three or four at the base are so

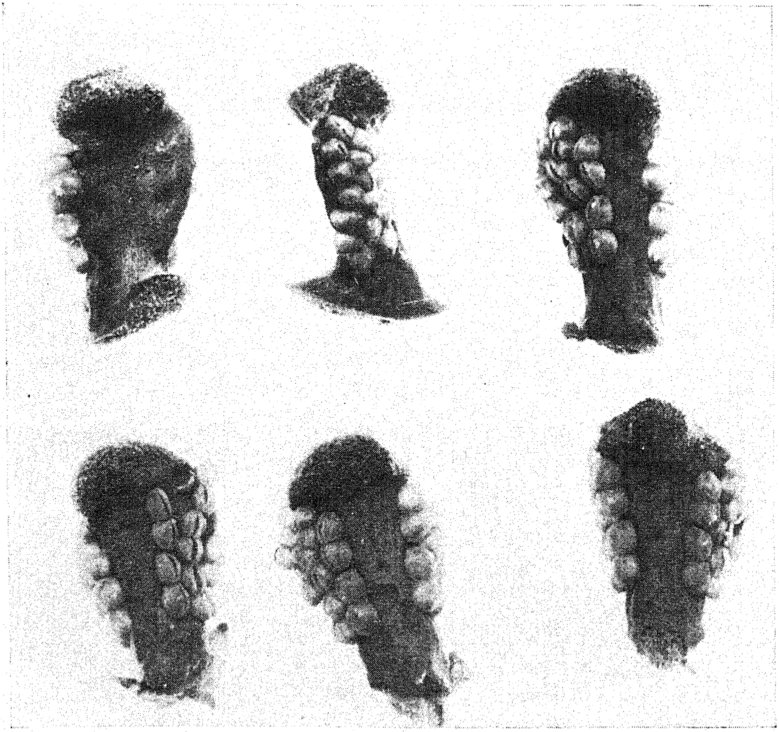

FIG. 2.—*Zamia monticola:* microsporophylls in various positions, all showing characteristic sori of two sporangia; middle sporophyll in top row shows one sorus with three sporangia; negative by C. Y. CHANG.

scattered that there is no indication of pairing; and the rest are intermediate between the alternate and the paired condition. The larger leaflets are 24–26 cm. in length, and 3.5–4 cm. in width. The lowest leaflet is sometimes as small as 7 cm. in length and 1.2 cm. in width, but there is no tendency to extreme reduction.

The number of veins, 1 cm. from the base of the leaflet, is about fifteen; and the widest part, about thirty. As the leaflet tapers from

the widest part to the tip, the veins are lost in the margin, usually with no tendency to produce serration, but sometimes producing a distinct serration near the tip (fig. 3). Most of the leaflets are as

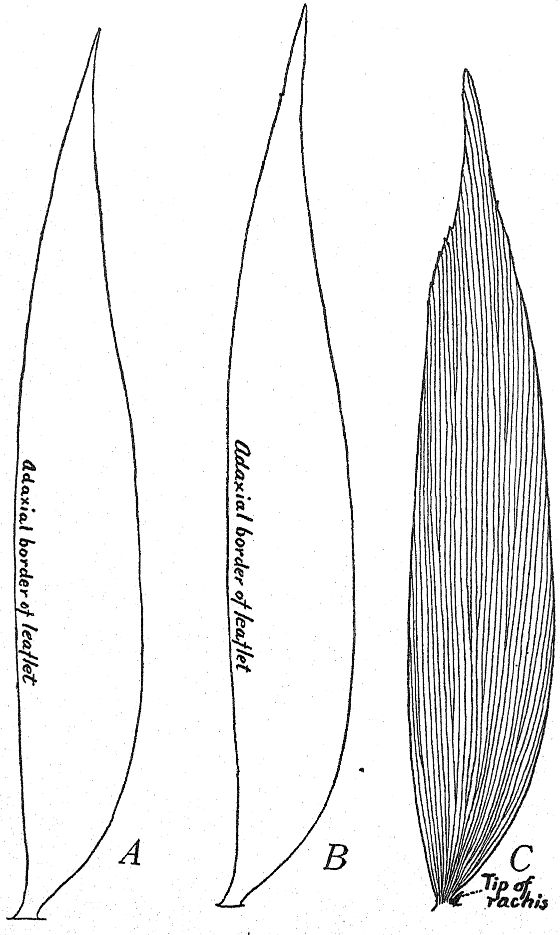

FIG. 3.—*Zamia monticola*, three leaflets: *A* (entire) and *B* (almost entire), below middle of leafy portion; *C*, one of terminal pair, showing almost maximum serration and also venation; one-half natural size.

entire as those of *Ceratozamia*, a feature which might be a partial excuse for not noticing earlier that the plant is a *Zamia*. The leaflets on the lower half of the leafy region are practically free from serration, and so far as their contour is concerned, many of them might

be mistaken for *Ceratozamia*, also accounting for not noticing a plant of *Zamia* in a patch of *Ceratozamia;* but in practically every leaf some of the upper leaflets show serration (fig. 3).

With so many large leaves in a crown, and crowns produced in such rapid succession, it is natural that the stem should develop rather rapidly. At present, the trunk above ground is 21 cm. high and 14 cm. in diameter. The armor is well developed on the part above ground, with no indication of being reduced, as in the tuberous species of the genus.

The characters described are summarized in the following diagnosis:

Zamia monticola, sp. nov.—Male cones oblong-ovoid, 12–16 cm. long; peduncles 10–17 cm. long, much expanded at base of cone and between horizontal and erect position. Microsporophylls hexagonal in surface view, sporangia in two widely separated groups with 10–16 sporangia in a group, sporangia mostly in pairs. Female cones not known. Leaves numerous, 20 or more in a crown, 1.3–1.7 m. in length, lower part of petiole spiny, 30–34 leaflets with upper 20 approximately in pairs and the rest scattered; larger leaflets 24–26 cm. long and 3.5–4 cm. wide, mostly entire but some serrate near the tip; about 30 veins in the widest portion. Stem arborescent.—On mountain side near Jalapa, Mexico, opposite the extinct crater of Naolinco.

Zamia sylvatica

This species was secured in September 1910 from the dense forest across the Papaloapan River south of Tuxtepec. The distance was probably not more than five miles beyond the river. A few miles farther on, *Dioon spinulosum* occurs in great abundance. It also has rather large leaves for a *Zamia*, those of this specimen reaching 1 m. in length, but produced sparingly, with only 2–4 in a crown, and coming at long intervals. At present the plant has only one leaf (fig. 4). The petioles are quite free from spines.

The next spring after the plant was set out, it produced two leaves; two more appeared in April 1915, and a crown of four leaves came in 1920. All of the leaves are about 1 m. in length. The average number of leaflets is about 34, and nearly all of them are more or less paired (fig. 4). The largest leaflets are 32–34 cm. long, and

2.3–2.6 cm. wide. The number of veins at the widest part of the leaflets varies from 40 to 46. The leaflet is entire below the middle, but is finely and sharply serrate near the tip (fig. 5). In the spring of 1921, four leaves and also a female cone appeared, which, without pollination, reached a length of 10 cm., with a peduncle 11 cm. long (fig. 6). Before taking this negative, some of the top soil was removed, and it became evident that the plant was a branching specimen, with two leaves on one branch and two leaves and the cone on the other.

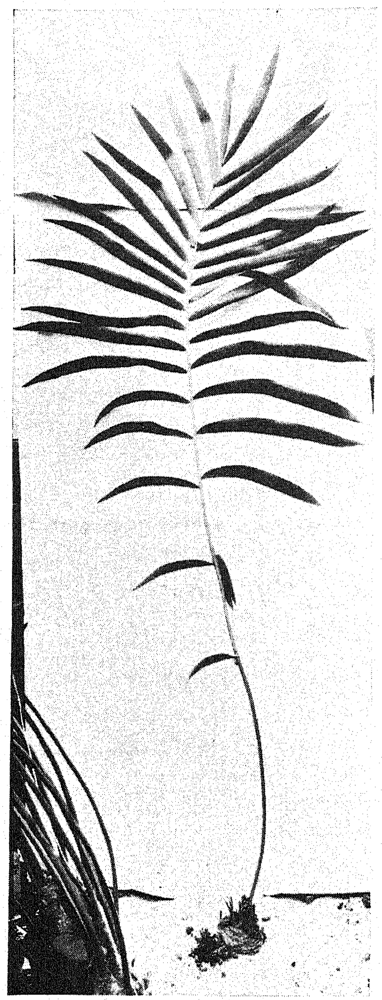

FIG. 4.—*Zamia sylvatica:* plant with one leaf, 1 m. in length, in University of Chicago greenhouse; negative by C. Y. CHANG.

The sporophylls, while spiral, show a longitudinal arrangement to an unusual degree. In surface view, the transverse ridge is a little above the middle of the sporophyll, and extends entirely across. From the ridge, the sporophyll slopes toward the top and the bottom, but about 1 mm. from the bottom the angle changes, and the final millimeter is parallel with the axis of the cone (fig. 6). Even in the illustration, which is about two-thirds natural size, the parallel portion can be distinguished. If pollination and fertilization had taken place, the cone would undoubtedly have been larger. All the ovules were abortive. Male cones are unknown. The larger branch of the stem is 8 cm. in diameter, and the smaller, which bears the cone, measures 5 cm.

The following diagnosis, while lacking the male cone and the seed, will serve to identify the species:

Zamia sylvatica, sp. nov.—Female cone cylindrical, 10 cm. or more in length, 5.5 cm. in diameter. Scales in surface view hexagonal

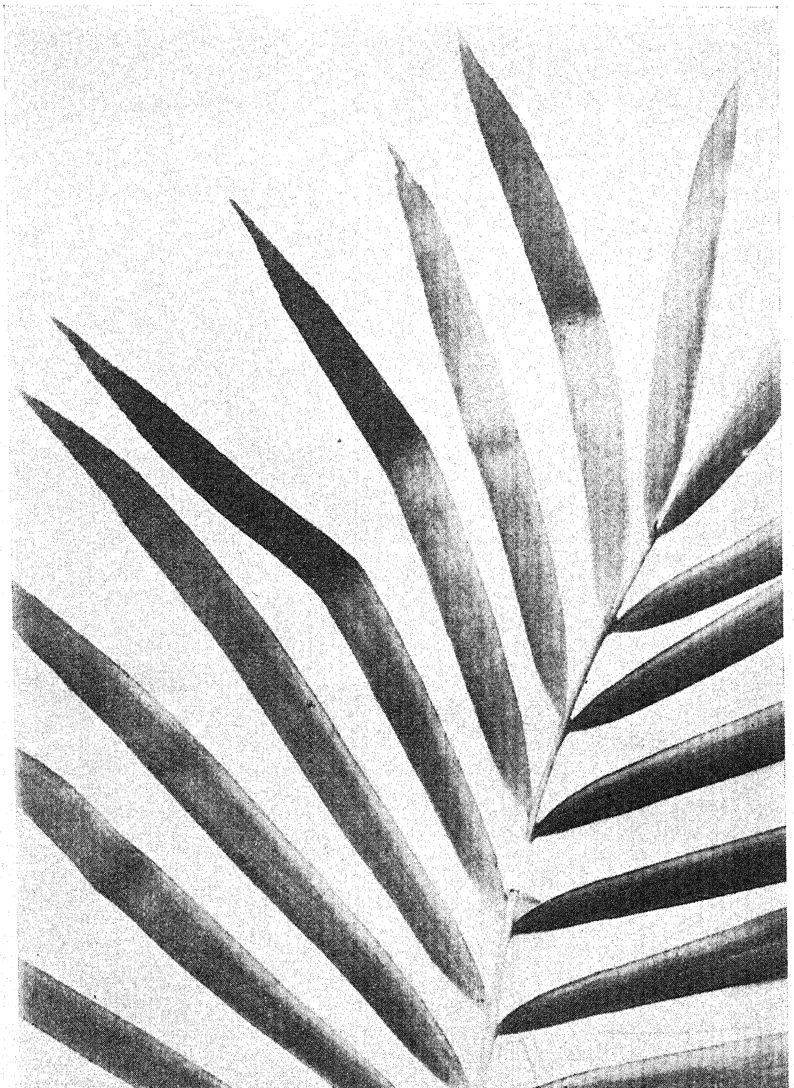

Fig. 5.—*Zamia sylvatica:* upper part of leaf, showing fine but sharp serration near tips of leaflets; negative by C. Y. Chang.

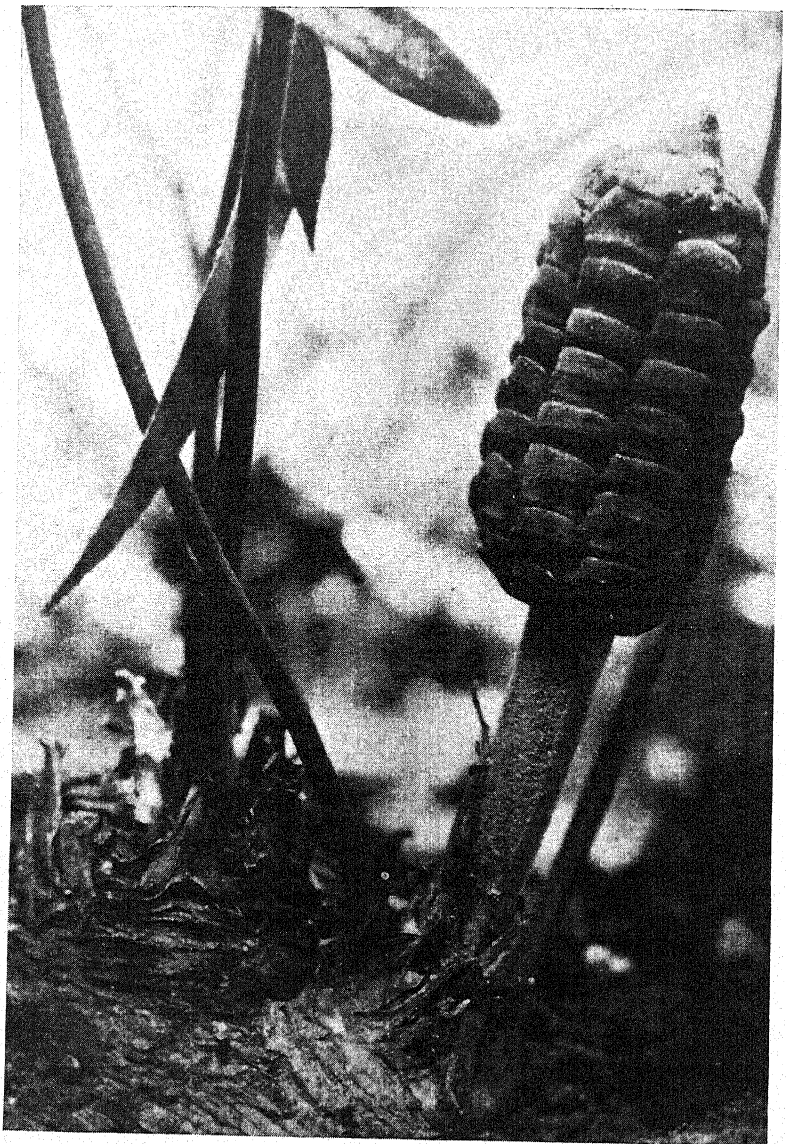

FIG. 6.—*Zamia sylvatica:* upper portion of branched plant; one branch bearing two leaves, and the other two leaves and a female cone; cone 10 cm. in length, with peduncle nearly as long; negative by P. J. SEDGWICK.

with transverse ridge extending entirely across, and the lower millimeter of the lower part of the sporophyll parallel with the axis of the cone. Male cone unknown. Leaves about 1 m. in length, with 2–4 in a crown. Petiole smooth. Leaflets about 32, approximately in pairs, the longest leaflets 32–34 cm. in length and 2.3–2.5 cm. in width; number of veins in the widest part of the leaflet 40–46. Leaflets entire below the middle, finely and sharply serrate toward the tip. Stem subterranean.—In the forest about five miles south of the Papaloapan River at Tuxtepec, Mexico.

It is very probable that a careful examination of the regions cited for these two species of *Zamia* would yield other cycads, besides undescribed plants in other groups.

UNIVERSITY OF CHICAGO

BRIEFER ARTICLES

THE AUXIMONE QUESTION

It is more than ten years now since BOTTOMLEY[1] conceived that plants are unable to continue healthy and normal growth in purely inorganic cultures, but require certain accessory organic substances, for which he proposed the name *auximones*.[2] For some years he and his pupils pursued this principle experimentally, and established it to their own satisfaction for cultures of *Lemna*,[3] only MENDIOLA[4] dissenting openly from their view.

In the summer of 1922 the writer undertook to test the correctness of this theory, and carried on certain experiments. These were never reported, because they were felt to be incomplete, and it was hoped to complete them. In the past year, however, two papers have been published dealing with this question, one of them giving some of the data for lack of which the writer's results were withheld from publication. Since interest is again aroused at this time, and since the writer's experiments seem to make a timely addition to our present published knowledge, it is deemed wise to offer now the results of this former work, together with a critical review of the whole situation to date.

BOTTOMLEY used pond water and the standard nutrient solutions of KNOP and DETMER as culture media, and was unable to maintain the health of *Lemna* and other water plants in these two artificial media. The fact that SHIVE[5] had secured notably better results with wheat grown in a nutrient solution of his own devising than in Knop's solution, suggested that BOTTOMLEY was not supplying a properly balanced nutrient solution, the lack of inorganic balance being made good by the organic additions termed "bacterized peat extracts." Accordingly, a series of cultures

[1] BOTTOMLEY, W. B., Some accessory factors in plant growth and nutrition. Proc. Roy. Soc. Lond. B **88**:237–247. 1914.

[2] ———, A bacterial test for plant food accessories (auximones). Proc. Roy. Soc. Lond. B **89**:102–108. 1915.

[3] ———, The effect of auximones on the growth of *Lemna minor* in nutrient solutions. Proc. Roy. Soc. Lond. B **89**:483–506. 1917 (SAEGER gives very complete citations).

[4] MENDIOLA, N. B., Variation and selection within clonal lines of *Lemna minor*. Genetics **4**:151–182. 1919.

[5] SHIVE, J. W., A 3-salt nutrient solution for plants. Amer. Jour. Bot. **2**:157–160. 1915.

of *L. minor* was tried with Series 1 of the 3-salt nutrient solutions for water cultures advocated by the National Research Council,[6] using Shive's Best for Wheat as control.[7] Of the Series 1 solutions, no. 7 gave the best growth, but the control far surpassed it. In neither case was there any sign of loss of vigor and healthiness during the five weeks'

TABLE I

Inorganic media, 36 days		Inorganic+organic media, 20 days		
Culture no.	Percentage gain	Organic addition	ppm	Percentage gain
1	1500	Arabinose	200	550
2	1350	Alanine	65	170
3	1200	Asparagine	200	200
4	1100	Caffeine	65	0
5	1150	Dextrose	200	600
6	900	Diphenylamine	65	300
7	1750	Galactose	200	600
8	1000	Phenylhydrazine	65	0
9	1100	Piperine	65	750
10	1150	Pyridine	200	460
11	1400	Urea	200	160
12	1450	Urea	150	220
13	1400	Urea	100	280
14	1150	Urea	50	730
15	1000	Control	....	900
16	1900	Control	....	940
17	1150	....	....	....
18	1250	....	....	....
19	1500	....	....	....
20	1400	....	....	....
21	1200	....	....	....
Control	2750 (about 950 in 20 days)			

duration of the experiment, whereas Bottomley reported unhealthiness from the start with Detmer's solution. The left side of table I presents in full the results of this experiment, the increase in number being corrected for size, so that the figures represent practically increase in area. Culture 16 shows a higher figure than no. 7, but the plants in it were in not nearly such healthy condition nor so large. Thus it seemed almost certain that the previous failures to maintain normal growth in purely inorganic

[6] Livingston, B. E. (ed.), A plan for cooperative research on the salt requirements of representative agricultural plants. 2d. ed. Baltimore. 1919.

[7] It has been found that owing to an error in the directions of the National Research Council bulletin, the control solution used in the writer's experiments was three and one-half times as dilute as Shive's Best, which was supposed to be the control.

media were due to a lack of proper physiological balance, and not to specific need of hypothetical accessory organic factors.

The next step was to determine what would be the effect of the addition of small quantities of organic matter to a physiologically balanced inorganic nutrient solution. Instead of the unknown factors added in the bacterized peat extract, various known compounds were used in low concentrations. These compounds, their concentration (in parts per million), and the results of their addition to the control used in the inorganic experiments are shown on the right side of table I. In no case was growth so good in the presence of these organic additions as in their absence, although the small quantities added can hardly have exercised any specific toxicity, in the case of the sugars at least.

Turning now to a consideration of the work of previous writers, we find that MENDIOLA noted that a modified Pfeffer's solution gave good growth of *Lemna*, but the observation was rather an incidental one only. CLARK and ROLLER[8] obtained even better growth in certain inorganic media than did BOTTOMLEY with Detmer's solution plus bacterized peat extract, the rate of reproduction being maintained at a high value in their experiments, while in his it was initially high but soon fell off. They employed 125 cultures, using this number of various combinations and concentrations of the salts of Series 3 of the National Research Council's bulletin. Unfortunately they state only that they found several combinations which were satisfactory, and do not state the actual constitution of these solutions, so that their work cannot readily be made the basis of further investigation.[9] They seem to show very conclusively, however, that organic matter is unnecessary in nutrient solutions if a proper physiological balance of inorganic salts is maintained.

Both these workers and the writer failed to employ the media used by BOTTOMLEY, and it was for this reason that the writer felt his own work to be inconclusive and incomplete. Very recently, however, SAEGER[10] has published an account of experiments in which the media used by BOTTOMLEY were compared with others, in this case simply various dilutions

[8] CLARK, N. A., and ROLLER, E. M., "Auximones" and the growth of the green plant. Soil Sci. 17:193–198. 1924.

[9] Since the preceding was written, CLARK has published a paper (CLARK, N. A., The rate of reproduction of *Lemna major* as a function of intensity and duration of light. Jour. Phys. Chem. 29:935–941. 1925) in which he gives the exact composition of one of the solutions found satisfactory.

[10] SAEGER, A., The growth of duckweeds in mineral nutrient solutions with and without organic extracts. Jour. Gen. Physiol. 7:517–526. 1925. (This article has a very complete bibliography appended, especially of BOTTOMLEY's work.)

of these same. With ten times dilutions of both Detmer's and Knop's solutions, excellent growth was obtained of a species nearly related to that used by Bottomley, while his results were confirmed by the poor growth and sickliness of the plants grown in the normal strength of these solutions. Here again there is demonstrated the lack of need of organic substances in media.

Using the Knop/10 solution, Saeger examined the effect of small quantities of autolyzed yeast and of peat extract, and noted in both cases considerable increase of growth, although the same was true if the natural pond water was substituted for the artificial solution and these organic substances of unknown nature were added thereto. No explanation, of course, is possible for the catalytic effect of these substances, and their unknown nature only aggravates the difficulties already presented. It is probable that there are other inorganic media which would give even better growth than the diluted Knop's solution, but since Saeger used a different plant in his cultures it is not possible to compare his results with any others.

In view of the results of these four independent sets of experiments, all of which refute Bottomley's theory completely, and point to lack of physiological balance as the difficulty which led to its proposition originally, it seems that we may well drop from the literature the term *auximones*. Whether in a physiologically balanced inorganic nutrient medium it is possible to stimulate growth still further by the addition of organic substances is beside the question, since auximones were hypothecated as absolutely indispensable for the continued healthy growth of a plant, and not simply as capable of increasing growth. The work of Clark and Roller and of the writer would seem to indicate that such additions will not increase the rate of growth, and while Saeger has obtained some increase, it must be remembered that he did not assure himself of having the best inorganic medium possible initially, and that his unknown extracts also contained inorganic as well as organic matter. It is, of course, impossible to compare directly the results of two different experimenters quantitatively, because of the great differences in the environmental conditions and in the hereditary factors involved in the two cases. We await with interest further experimentation in which these various difficulties shall be eliminated; but whatever the results, auximones as conceived by their namer do not exist.—H. S. Wolfe, *Department of Botany, University of Chicago.*

CURRENT LITERATURE

BOOK REVIEWS

Photosynthesis

Many investigators have attempted to obtain knowledge of how the plant utilizes radiant energy in the manufacture of organic compounds. A useful summary of the literature of this problem has been prepared by STILES,[1] who has not neglected any important section of the world's work dealing with this subject. The book is in no sense a mere revision of the earlier review by JØRGENSEN and STILES. It presents a more general view of the developments since the days of PRIESTLEY and INGEN-HOUSZ, but preserves the admirable critical spirit in handling all the recent contributions to our knowledge of this difficult problem.

The introductory chapter gives a rather brief glimpse of the history since the phlogiston idea of carbon was abandoned. This chapter could have been made more extensive with profit to the students of today. Following the introduction, there is a short discussion of the system involved in the food manufacturing cells and tissues. The pigments, chlorophylls, and carotinoids are described, and methods of demonstrating photosynthesis are presented. Beginning with chapter v, the author takes up the main questions, methods of measurement, intake of CO_2, influence of internal and external conditions on the process, the products of synthesis, utilization of energy, the mechanism as a chemical process, and the relation of photosynthesis to other processes of life. The concluding remarks summarize the progress made, and point the way to larger advancement.

With reference to the work on the mechanism of photosynthesis, the present situation is summarized by quoting SACHS' statement: "Whether it is right to claim . . . . formic acid or some other member of the formyl group as the first product of assimilation on account of its simple constitution, I hold as at least very questionable; and it has hitherto been proved by nothing." This quotation, from the nineteenth chapter of *Lectures on the physiology of plants* (1882), may be as truly applied to the more recent hypotheses suggested to account for the conversion of carbonic acid into sugar in green plants.

The book presents a bibliography of nearly 900 citations, which is a great convenience to the student who desires to consult the original sources. It is written in simple, readable style, and avoids the mass of details which would only obscure the picture.—C. A. SHULL.

[1] STILES, WALTER, Photosynthesis. 8 vo. pp. viii+268. *figs. 15.* London: Longmans. 1925.

Textbook of plant physiology

A concise textbook of plant physiology has been written by LEPESCHKIN,[2] who treats the subject from the physico-chemical point of view. The introduction discusses the peculiarities of living matter and physiological phenomena, colloidal behavior of living protoplasm, external and internal conditions of life, irritability, mechanism, and the classification of physiological phenomena in plants.

The discussion then proceeds in three sections: metabolism, growth, and movement. Metabolism is considered in five chapters: first the general physico-chemical basis of metabolism, then chapters on water in the plant, minerals, organic compounds, and respiration. Two chapters on growth present the general physico-chemical basis of cell growth and reproduction, and a descriptive and explanatory treatment of growth phenomena, as influenced by temperature, light, humidity, stimulation, nutrients, and toxins. This chapter includes some discussion of correlation and transplantation of tissues. The section on movements is similarly divided into a general discussion of the physico-chemical basis for the phenomena of movement, and a descriptive and explanatory discussion.

No attempt has been made to summarize the entire literature of plant physiology, but to present a concise, well organized statement, citing only the most important literature, the outstanding contributions. The author succeeds remarkably well, in a book of such small compass. Some may object to the small space given to the growth phenomena, which occupy only 68 pages, and to the anticlimax provided by the section on movements. Notwithstanding these matters of emphasis and organization, the author has given us a useful brief summary of this fundamentally important science.—C. A. SHULL.

Cyclopedia of horticulture

The great success of BAILEY'S *Cyclopedia of horticulture* is well attested by its numerous reprints and revisions. There has recently appeared a reprint of the entire Cyclopedia, in three volumes instead of the customary six volumes of previous editions.[3] The bulk of the volumes has been very much reduced by the use of thinner paper, which, however, is strong and easily turned. This edition of the Cyclopedia is very handsome and satisfactory, and is much the most attractive and easily usable of the long series which started a quarter of a century ago.—H. C. COWLES.

NOTES FOR STUDENTS

Carbohydrates in nitrogen metabolism.—Etiolated seedlings differ in their power to utilize ammonia as a source of nitrogen. High carbohydrate seedlings, barley for instance, easily form asparagin from ammonium salts, while some of

[2] LEPESCHKIN, W., Lehrbuch der Pflanzenphysiologie auf physikalisch-chemischer Grundlage. 8vo. pp. vi+297. *figs. 141.* Berlin: Springer. 1925.

[3] BAILEY, L. H., The standard cyclopedia of horticulture. Vols. I–III. pp. xxiv+639. *pls. 120 (24 colored). figs. 4000.* New York: Macmillan Co. 1925.

the legumes cannot use ammonia compounds unless $CaCO_3$ is present to keep down acidity. The yellow lupine among legumes has been found by PRIANISCHNIKOW[4] to be exceptional, in that even when the carbonate is present it cannot use ammonia as a source of nitrogen. Instead of building up proteins, it stores the ammonia as such in its tissue, and such proteins and amides as have already been formed are converted into ammonia, so that the stored ammonia represents more than that absorbed. The plants exhibit ammonia toxicity under these circumstances. He finds that this peculiar behavior is not hereditary, but depends upon internal conditions of nutrition. If the etiolated seedlings are fed on soluble carbohydrates, or if active green seedlings are used in light, so as to convert them from low to high carbohydrate seedlings, in either case the lupines can use the ammonia to build asparagin. There is no accumulation of ammonia in the tissue. In other words, the lupines behave like barley when like barley they have a high carbohydrate supply. Again, if the barley seedlings are starved by exhaustion of the reserves before use, or if the endosperm is cut away, they lose their power to form asparagin from an ammonia supply, and behave like lupines.

PRIANISCHNIKOW considers that the nitrogen metabolism is a reversible chemical reaction, running from proteins and amino acids by oxidation and secondary synthesis to amides, and from these by further oxidation to ammonia, especially in the absence of sufficient carbohydrate. When sufficient carbohydrates are present the process is reversed, and ammonia can be utilized to form amides, which then build up into amino acids and proteins. In such a system, changing the quantity of carbohydrate reverses the whole process, and the amount present will determine whether the plant can build proteins, or whether it will store nitrogen as ammonia. Ammonia is considered the first step in synthesis, the last in the disintegration of the proteins. The same principles apply when nitrates are the nitrogen source, only that the nitrogen must first be reduced to the condition of ammonia. The work throws interesting light on the carbohydrate-nitrogen ratio which has assumed importance in the literature in recent years.—C. A. SHULL.

Japanese vegetation.—The Island of Yezo lies to the north of Honshu, the main island of Japan, and between 41° 24′ and 45° 31′ north latitude. It is therefore in the same latitude as northern United States, and a recent description of its vegetation[5] serves to emphasize the similarity of the plant communities of the two regions. The forests are said still to cover more than half the whole area, and are made up of broad leaved deciduous, conifer, and mixed types, the conifers being more plentiful toward the north.

The largest trees are *Populus Maximowiczii*, *Cercidophyllum japonicum*, and

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[4] PRIANISCHNIKOW, D., Über den Aufbau und Abbau des Asparagins in den Pflanzen. Ber. Deutsch. Bot. Gesells. 40: 242–248. 1922.

[5] KUDO, YUSHUN, The vegetation of Yezo. Jap. Jour. Bot. 2: 209–292. 1925.

Quercus grosseserrata, while among the most widely distributed species are *Salix jessoensis*, *Ulmus japonica*, *U. laciniata*, *Betula japonica*, *B. Ermani*, *Morus bombycis*, *Alnus hirsuta*, *Prunus serrulata*, *Tilia japonica*, *T. Maximowicziana*, *Acer pictum*, and *Fraxinus pubinervis*. In addition there is a group of genera such as *Magnolia*, *Styrax*, *Picrasma*, *Ostrya*, *Carpinus*, and *Pterocarya*, which occur but are more characteristic of the southern main island. Among the conifers, some of the more abundant are *Pinus pentaphylla*, *Abies sachalinensis*, *A. mayriana*, *Picea jezoensis*, *P. Glehni*, *Larix dahurica* var. *kamtschatica*, and *Thujopsis dolabrata*. Notes are given on the habits of these and other tree and shrub species. The number of woody plants is 256, including 24 woody climbers, and the entire vascular flora includes 1629 species. The island is divided by a central depression into an eastern and a western region. The latter is much the smaller, being only one-sixth of the whole, and presents a hilly and mountainous surface, but with no peaks above 5000 feet. The eastern division on the other hand has broad plains and higher mountains, culminating in Mt. Taisetsu, 7500 feet. With an unusual precipitation ranging from 80 to 116 cm., the six botanical districts exhibit plant associations and formations very similar to those of eastern North America. These districts are: (1) southwestern Yezo, dominated by birch forests and showing a close affinity to northern Honshu; (2) southeastern Yezo, showing southern affinities but dominated by fir and spruce forests; (3) central Yezo, with an abundance of bogs and alpine meadows, but with deciduous forests on the plains becoming mixed with conifers on the mountain slopes; (4) eastern Yezo, with many swamps in the western portion of the district, including extensive associations of *Phragmites communis* and forests of *Abies sachalinensis* and *Picea Glehni* in the west; (5) Province of Kitami along the northeast coast, similar to District 3 but with more northern affinities; and (6) southern Kurile Islands, similar to District 4 but with decidedly fewer species.

The floristic affinity of Japan and New England, so excellently described by GRAY, is made still more evident by the analysis here given by KUDO. His list of species gives not only their occurrence in Yezo and elsewhere in Japan, but also in many other countries. Thus the relationship of the flora of Yezo and other regions is shown by the fact that 79 per cent of its species are common to Honshu, and over 38 per cent are also common to Korea, Kyushu, Shikoku, China, Manchuria, and Saghalien; while Siberia, Europe, and North America each have more than 26 per cent of the species now recorded for the island.

An examination of orders and genera shows that the Pteridophytes include 30 genera with 98 species, the Taxaceae 2 genera of 1 species each, the Pinaceae 6 genera with 11 species, and the angiosperms 122 families, 559 genera, and 1518 species. Some of the larger families, in the order of size and number of species, are Cyperaceae 142, Gramineae 117, Compositae 116, Rosaceae 73, Liliaceae 64, Orchidaceae 61, Ranunculaceae 55, Cruciferae 44, Umbelliferae 39, Labiatae 38, Ericaceae 38, Leguminosae 38, Caryophyllaceae 36, Polygonaceae 36, Scrophulariaceae 35, Saxifragaceae 35, and Violaceae 32.—GEO. D. FULLER.

A genetic, ecologic, and taxonomic study of Hemizonia.—A recent paper by BABCOCK and HALL[6] marks a definitely forward movement in taxonomy, because it approaches the question of species by a combination of methods of different fields. It has long been the opinion of the reviewer that the concept of species is not a purely taxonomic matter, but that other fields of botany also must contribute to its understanding. In 1836 DE CANDOLLE first described *Hemizonia congesta* as the first species of a new genus which he established. At the same time he recognized and described a second species. Prior to 1884 four other species were recognized by various taxonomists. In 1884 the total number of species was reduced to three by ASA GRAY. Since that time two more have been described. By the combination study of the authors, the species now have been reduced to one, namely, *Hemizonia congesta*. The other so-called species are found to be only subspecies. This conclusion is not due to a hasty study but to one that has covered years. HALL began his taxonomic and ecologic study of *Hemizonia* in 1912, and BABCOCK began his genetic study in 1915. Nor does the conclusion mean a shifting of species concepts. It means rather access to a greater abundance of material, accompanied by adequate field study and experimentation. The six subspecies have innumerable forms, some of which are genetic and can be maintained by selection. The subspecies are roughly correlated with geographic distribution. There are no differences in the number or individuality of the chromosomes. Artificial hybrids are easily produced and sometimes they develop supposed natural hybrids. Since the inheritance is Mendelian, the evolution of the subspecies is thought to be by mutation. Natural selection of favorable mutations accounts for the existence of five of the subspecies, and the geographic distribution of three subspecies indicates the importance of isolation in establishing them. It is to be hoped that the future will see many more joint investigations of this sort. Whether or not such studies will radically decrease the number of so-called species is a minor matter, although most botanists regret the indefinite multiplication of "species." The real value of contributions of this sort is to put taxonomy on an absolutely sound and scientific basis, and in line with modern experimental science.—H. C. COWLES.

An ecological study in the Himalayas.—An interesting paper has just appeared from the hands of DUDGEON and KENOYER[7] on the ecology of Tehri Garhwal, which is a state of the western Himalayas with an altitudinal variation of over 20,000 feet. Three great zones of vegetation are recognized, corresponding to three climatic zones, essentially as recognized in SCHIMPER'S *Plant geography*. The lowest of these zones is occupied by the tropical monsoon forest which

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6 BABCOCK, E. B., and HALL, H. M., *Hemizonia congesta*, a genetic, ecologic, and taxonomic study of the hay-field tarweeds. Univ. Calif. Publ. Botany **13**:15–100. 1924.

7 DUDGEON, W. S., and KENOYER, L. A., The ecology of Tehri Garhwal: A contribution to the ecology of the western Himalayas. Jour. Ind. Bot. Soc. **4**:233–285. 1925.

extends to an altitude of 5000 feet. Above this are sclerophyll forests with broad leaved trees, with an altitude range from 5000 to 11,000 or 12,000 feet, depending upon circumstances. Finally, there are alpine formations in the higher stretches of the mountains. The monsoon forests, unlike some others of India, are dominated by species of *Bauhinia*, and the sclerophyll forests are dominated by various species of *Quercus*. Interesting forests of *Pinus longifolia* and *Cedrus Deodara* are also described. These are not regarded as parts of the climatic climax forest but rather as edaphic climaxes. The pine edaphic climax lies partly in the monsoon forest region and partly in the sclerophyll forest, whereas the deodar edaphic climax lies entirely within the latter region. In the timber line areas occur *Betula utilis* and *Abies Webbiana*.

The latter portion of the paper is taken up with a discussion of the human factors and their influence on the native vegetation. On account of the very dense population, the pressure of human factors is enormous. In the lower altitudes there is found a relatively scant development of the natural formations. The climax vegetation is almost destroyed by man, following which still more disastrous effects are brought about by erosion. Especially serious is the complete destruction of the forest over considerable areas through logging operations, which are followed by severe erosion and the development to some extent of xerophytic pioneers. There is much burning of the dead vegetation during the dry spring, which serves to prevent forest regeneration, especially in the pine forests. These destructive human activities have been taking place for a long time, and seem to be getting worse rather than better.—H. C. COWLES.

Absorption of cations by protoplasm.—SCARTH[8] has studied the penetration of cations into living protoplasm. A physiological reaction was used as a test of penetrability. This consisted of the contraction along their long axes of the chloroplasts and cytoplasmic strands of *Spirogyra*. Especially in the case of many bivalent and trivalent cations, there was observed a retarding of penetration as time proceeded, this flattening out of the curve being more marked in the case of the higher concentrations. Self-antagonism of ions is given as one probable factor in causing this decrease in the rate of penetration. The mechanism of self-antagonism is regarded as the same as antagonism between ions, the seat of the reaction appearing to be the lipoid exterior of the protoplasm. So two reactions of the cell control entrance of the ions, one tending to bring about active absorption of the ion, the other tending to exclude the ion. The sensitiveness of both of these reactions increases with the valency of the ion. As the atomic weight of the ion increases, the factor bringing about active absorption also increases. In the case of the heavy metals, this factor also increases inversely with the electrolytic solution pressure of the ions. This is not true of the other ions. The order of the ions as regards initial penetrability is the same in

[8] SCARTH, C. W., The penetration of cations into living protoplasm. Amer. Jour. Bot. 12:133–148. 1925.

general as their order arranged on the basis of their physiological activity. The author recognizes that it is impossible at present to explain the physiological series of ions by any single physico-chemical law, and so describes the absorption of ions by the protoplasm and their passage through it as a "vital function." He realizes, of course, that this does not explain the process, and states that adsorption seems to be one factor involved, the absorption curve resembling an adsorption curve. That absorption is not a simple diffusion process, is shown by the fact that the rate is not proportional to the diffusion gradient. Problems connected with the absorption of ions by protoplasm are among the hardest and most important confronting plant physiology, and deserve much more work in the effort to solve them.—S. V. EATON.

Relation of light to growth.—ADAMS[9] reports the results of experiments on the relation of light to growth. Under continuous electrical illumination the castor bean was the only species worked with which completed its entire life cycle from seed to seed, although several other species flowered. Some species made better growth in daylight, others in daylight supplemented with electrical illumination. Experiments on the duration and intensity of light showed that two hours' exposure to light at midday gave as good results as three hours' exposure during either the morning or the afternoon. Results of experiments are reported also on the interrelation between temperature and duration of exposure to light, and on the effect on growth of interposing a screen of colorless glass.

Using the tomato, pepper, and nasturtium, DEATS[10] obtained results in agreement with those of GARNER and ALLARD. The amount and rate of growth in each species were proportional to the length of the daily exposure to light, but the requirements for flower formation differed in the different species. The tomato behaved as a long-day plant, the pepper as a short-day plant. Preliminary work with the nasturtium indicated that it too is a short-day plant. Anatomical studies were made on the effect of the length of illumination on bast and xylem development, on cork development, on size and thickness of leaves, and other features. Starch was the only compound determined, the amount of this being determined in a qualitative way by microchemical means. It was found to be greater in the long-day plants. The short-day plants were small, pale, and yellowish, and contained little starch. They had been grown with a good supply of nitrates. Reference is made to unpublished work of ELTINGE, who, working with the same species but varying the nitrate supply, much as did KRAUS and KRAYBILL, found that the plants grown without nitrogen were also undeveloped and of a poor color. They contained much starch

[9] ADAMS, J., Some further experiments on the relation of light to growth. Amer. Jour. Bot. 12:398–412. 1925.

[10] DEATS, M. E., The effect on plants of the increase and decrease of the period of illumination over that of the normal day period. Amer. Jour. Bot. 12:384–394. 1925.

however. The author points out that the two sets of experiments illustrate two of the conditions mentioned by KRAUS and KRAYBILL as causing poor vegetation and non-fruitfulness, namely, high nitrate supply and low carbohydrates, or low nitrate supply and conditions of abundant carbohydrate production. The author recognizes that fuller chemical analyses are needed in experiments of this kind. The general conclusion of the work is that different lengths of day influence the development of plants by changing the carbohydrate-nitrogen ratio.—S. V. EATON.

Sex characters.—Two papers have recently appeared from the California Agricultural Experiment Station dealing with problems of sex. ROBBINS and JONES[11] have discovered some interesting secondary sex characters in *Asparagus*. Staminate plants express their sex earlier in life than do pistillate plants. The average height of the first flower-bearing shoot of staminate plants is not so great as that of the pistillate plants. There is also a marked difference in the green weight of the tops, that of the staminate plants being considerably greater than that of the pistillate. Other differences were noted also as to yield and weight of spears, the staminate plant exceeding the pistillate in both particulars.

ROSA[12] has investigated spinach, which is a dioecious, wind-pollinated plant. He distinguishes the sex arrangements under four categories: "extreme males," bearing only staminate flowers with reduced or even suppressed leaves on the flower branches; "vegetative males," bearing only staminate flowers, but with well developed leaves on the flower branches; "monoecious" plants, bearing staminate and pistillate flowers in variable ratios in the same cluster, and with fully developed leaves on the flower branches; and "female" plants, bearing only pistillate flowers and fully developed leaves. In consequence, he calls spinach a "tetra-morphic" plant, although there are intergrading forms. He finds that environment has no effect in determining these types, and therefore the differences seem to be due to genetic factors. Tested in several ways, as influence of rich and poor soils, of shade and light, of early and late planting, of mutilation, no response in sex expression was discovered.—J. M. C.

Evolution of Vernonia.—GLEASON[13] has made a comparative study of the North American species of *Vernonia* in reference to their relationships and distribution. The so-called sections are remarkably unequal in their representation, three of them including only a single species each, and one of them represented by 120 species. He has discovered that characters which are held to represent primitive conditions in one group may indicate advanced evolution

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[11] ROBBINS, W. W., and JONES, H. A., Secondary sex characters in *Asparagus officinalis* L. Hilgardia 1:183–202. 1925.

[12] ROSA, J. T., Sex expression in Spinach. Hilgardia 1:259–274. 1925.

[13] GLEASON, H. A., Evolution and geographical distribution of the genus *Vernonia* in North America. Amer. Jour. Bot. 10:187–202. *figs. 3.* 1923.

in another, and that such characters have no apparent relation to environment. In every case the groups which have the simplest morphological features occur to the south, while the more complex groups appear progressively farther toward the north. He concludes that evolution and migration have proceeded together.—J. M. C.

Physiological studies of Phytophthora.—LEONIAN.[14] has investigated the genus *Phytophthora* with the purpose of securing a less difficult and more reliable taxonomy. The specific distinctions have been so limited and variable as to make the segregation of species very unreliable. Cultures were made of 53 strains, representing most of the described species, and responses to various media noted. As a result, these responses enabled the investigator to recognize specific reactions, and to construct a physiological key. *Pythiacystis citrophthora* has been transferred to *Phytophthora;* a number of so-called species have been merged in *P. omnivora;* and *P. pini* has been described as a new species which occurs on the roots of *Pinus resinosa* in Minnesota. Another interesting result of this investigation was the study of saltation phenomena, the conclusions being that no new species or varieties have been produced in this way.—J. M. C.

Embryo sac of Moringa.—RUTGERS[15] has discovered an interesting situation in the development of the embryo sac of *Moringa*, a tropical genus indigenous in the Indies, but long cultivated also in the African and American tropics. The chalazal megaspore functions, and by two successive divisions two nuclei occur at each pole. The third division is restricted to one of the micropylar nuclei, resulting in a 5-nucleate sac. A normal egg apparatus is organized, and the second male cell fuses with the two chalazal nuclei to form the endosperm nucleus. This behavior has been reported heretofore only by TREUB for *Garcinia.*—J. M. C.

Leaf spot of maize.—DRECHSLER.[16] has discovered that a leaf disease of maize occurring in Florida and the Philippines is caused by a new species of *Ophiobolus*, which he desc1ibes tentatively as *O. heterostrophus*. A detailed description of its life history is given, and the conclusion is reached that the species of *Helminthosporium* "with straight subcylindrical conidial germinating laterally from end and intermediate segments constitute a natural group distinct from the group of species producing curved elliptical conidia germinating by two polar germ tubes." It seems that this disease has been confused with leaf blight, which also occurs in tropical and subtropical regions.—J. M. C.

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[14] LEONIAN, L. H., Physiological studies on the genus *Phytophthora*. Amer. Jour. Bot. **12**:444–498. 1925.

[15] RUTGERS, F. L., Embryo sac and embryo of *Moringa oleifera* Lam. Reliquia Treubianae III. pp. 5. 1923.

[16] DRECHSLER, C., Leaf spot of maize. Jour. Agric. Res. **31**:701–726. 1925.

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THE

BOTANICAL GAZETTE

May 1926

REVISION OF THE GENUS ISOSTIGMA LESS.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 346

EARL EDWARD SHERFF

(WITH PLATES XXIII, XXIV)

During the past fifteen years, in the course of a monographic study of the genus *Bidens*, my investigations frequently have reached into the genus *Isostigma*. While a small genus, containing as interpreted by me only eleven known species, it displays considerable range in growth habit and even technical characters of flowers and fruits. Indeed, the only reliable distinction between *Isostigma* and *Bidens* appears to rest upon the nature of the style branches. In *Isostigma* these are elongated and of uniform thickness; in *Bidens* they are usually, although not always (for example, *B. cosmoides* [Gray] Sherff) short, and usually have a swelling at or toward the distal end.

The literature for *Isostigma*[1] appears to begin with *Tragoceras peucedanifolium* Spreng., described in 1826 (Linn. Syst. Veg. Edit. XVI. 3:576). This was based upon a plant by SELLO from the State of Rio Grande do Sul, Brazil. In 1831, LESSING (Linnaea 6: 513) founded the genus *Isostigma*. To this he transferred SPRENGEL's earlier species and added three more species, *I. simplicifolium*, *I. speciosum*, and *I. crithmifolium*. All of these were based likewise upon SELLO's plants. GARDNER, in 1848 (Hook. Lond. Jour. Bot. 7:408) described a fifth species of true *Isostigma*, although he wrong-

[1] Except as to *Bidens megapotamica* Spreng., discussed under *Isostigma megapotamicum* (Spreng.) Sherff.

ly called it *Glossogyne brasiliensis*. In 1884, BAKER (Martius Fl. Brasil. 6^{III}: 238–242, *pl. 71. fig. 1*) presented a treatment of the genus which included two additional names, *I. stellatum* (referable to *I. brasiliense*) and *I. dissitifolium;* also a variety previously named by SCHULTZ BIPONTINUS (*I. speciosum* var. *Riedelii*), and which is to be regarded as a valid species, *I. Riedelii.* In his treatment of *Bidens* (*l.c.*) he erroneously included two more species which must be referred to *Isostigma*, *I. scorzoneraefolium* and *I. acaule.* Since BAKER'S time, KUNTZE (Rev. Gen. 3^{II}: 160. 1898) has described *I. Hoffmannii*, and HERZOG (Fedde Rep. Sp. Nov. 7:358. 1909) has published a description of *I. Herzogii* Hassl. In 1916, HASSLER (Fedde *l.c.* 14:277) published certain notes upon *Isostigma*, describing varieties that will receive mention under their respective species.

Since no monograph heretofore has been published for this genus, it has seemed to me advisable to bring together a treatment of all the species known so far. In former days, before contemplating the present revision, I examined many of the specimens in the larger European and American herbaria. As a rule, no record was saved of such specimens, and so they cannot be cited in the present revision. Recently, however, I have been lent considerable material expressly for this work. For the great kindness and courtesy shown me in this connection, I wish to express my sincere gratitude to the following botanists: Dr. JOHN BRIQUET, Director of the Botanical Garden of Geneva; Dr. H. DIELS, Director of the Berlin Botanical Garden; Dr. A. W. HILL, Director, and Mr. A. D. COTTON, Keeper of the Herbarium, at the Royal Botanical Garden of Kew; Professor BORIS L. ISSATSCHENKO, Director of the Botanical Garden of Leningrad (Hort. Petropol.); Professors H. LE COMPTE and PAUL DANGUY, of the Museum of Natural History, Paris; Mr. W. R. MAXON, Associate Curator of the United States National Herbarium; Dr. B. L. ROBINSON, Curator of Gray Herbarium; Dr. E. DE WILDEMAN, Director of the Botanical Garden at Brussels.

Photographs of most of the plants cited are in my private collection, and duplicates are in the Herbarium of the Field Museum of Natural History.

ISOSTIGMA Less., Linnaea 6:513. 1831.—Herbae perennes vel basi suffrutescentes, saepe e rhizomate lignoso crasso brevi vel elongato ramosoque, glaberrimae, caulibus simplicimis vel moderate ramosis. Folia infra plerumque numerosa et ad basim caulis plus minusve rosulata, supra pauciora et saepe reducta et etiam squamis non dissimilia, omnia nunc integra et linearia lanceolatave, nunc dissecta segmentis linearibus vel filiformibus, interdum valde elongatis. Capitula saepe magna speciosaque, plerumque heterogama radiataque, floribus ligulatis feminea, tubulosis hermaphrodita, rarissime radio deficiente homogama. Involucrum campanulatum, plerumque 2- rarius 3-∞-seriale, bracteis intimis 1-seriatis membranaceis subaequalibus, exterioribus (plerumque brevioribus) angustis, omnibus basi breviter connatis. Receptaculum planum, paleis scariosis planis vel concaviusculis flores tubulosos subtendentibus onustum. Corollae radii ligulatae, lamina patentes, apice 2-3-dentatae, nunc extra atro-purpurascentes et intus plus minusve albido-argenteae, nunc omnino luteae vel luteo-rubrae, saepe parvae; florum hermaphroditorum regulares, tubulosae, limbo ampliato, cylindraceo, 4- vel 5-fido. Antherae basi obtusae, subintegrae. Styli florum hermaphroditorum rami angusti, in appendices longas subulatas hirtas desinentes. Achaenia obcompresso-tetragona vel plana, linearia vel oblonga, faciebus striata, glabra vel sursum setulosa, margine nunc exalata nunc tenuia vel saepius anguste alata, apice truncata, aristis 2 rectis divaricatisve, longiusculis vel brevissimis, laevibus vel sursum scabridis coronata.[2]

CLAVIS SPECIERUM

Folia indivisa (nisi ad apicem breviter furcata)
  Folia oblanceolata, ad basim caulis (pedunculi) dense rosulata, apice 3–5-dentata . . . . 2. *I. acaule*
  Folia angustiora
    Folia subulata, ad basim pedunculi dense rosulata . . . . . . 1. *I. brasiliense*
    Folia latiora, partim caulina
      Flores flavi vel lutei . . . . 3. *I. scorzoneraefolium*
      Flores atro-purpurei
        Capitula solitaria

[2] Generic description based, with various modifications, upon that given by BENTHAM and HOOKER, Gen. Pl. 2:389. 1876.

Caulis versus apicem plerumque plurisquamatus; ligulis longis perspicuisque . . . . 10. *I. Riedelii*
Caulis versus apicem fere vel omnino esquamatus; ligulis disco subaequantibus . . . . 5. *I. simplicifolium*
Capitula in corymbis 3–4-floris . . . . 4. *I. Herzogii*
Folia divisa
Capitula discoidea, minuta, demum 7–9 mm. alta et 6.5–10 mm. lata 1. *I. brasiliense*
Capitula radiata, pansa ad anthesin 1–2 cm. alta et 2.5–3.5 cm. lata 6. *I. Hoffmannii*
Capitula plerumque radiata, majora
Pedunculus subrobustus, altus, ad apicem 4–5 mm. crassus et perspicue squamatus . . . . 11. *I. speciosum*
Pedunculus gracilior, ad apicem plerumque tantum 1–3 vel nullis squamis praeditus
Folia caulina plura segregata . . . . 8. *I. dissitifolium*
Folia ad basim caulis dense rosulata
Segmenta subulata 0.2–0.7 mm. lata . . . . 9. *I. megapotamicum*
Segmenta linearia 0.5–1.3 mm. lata . . . . 7. *I. crithmifolium*

1. ISOSTIGMA BRASILIENSE (Gardn.) Benth. and Hook. *ex* Ind. Kew. **2**:1240. 1893; *Glossogyne brasiliensis* Gardn., Hook. Lond. Jour. Bot. **7**:408. 1848; Walp. Ann. **2**:869. 1851–52; *Isostigma stellatum* Baker, Martius Fl. Brasil. **6**$^{\mathrm{III}}$:239. 1884; *I. microcephalum* Baker, *l.c.*—Pl. XXIII, figs. *n, o, p, q, r, s, t.*—Herba basi fruticulosa, 1–3 dm. alta, caulibus demissis, perspicue ramosis, ramis confertis, dense caespitosis. Folia ad basim pedunculi dense rosulata, nunc simplicia, strictiuscula, apice mucronata, 2.5–7 cm. longa et circ. 0.2 mm. lata; nunc ternatim biternatimve secta, plerumque 1.5–3 cm. longa, segmentis acerosis, apice mucronatis, (siccis) circ. 0.2 mm. latis. Capitula parva, discoidea, longe pedunculata, demum cum fructibus (aristis inclusis) 5–9 mm. altis et 4–10 mm. latis, pedunculis monocephalicis, strictis, erectis, usque ad 2.5 dm. longis, ad medium unisquamosis vel etiam supra basim foliis unijugis simplicibus vel compositis praeditis. Involucrum campanulatum bracteis omnibus adpressis, rigidulis, brunneis, exterioribus circ. 3 vel 4, parvis, lineari-oblongis, 1–1.5 mm. longis; interioribus late lanceolatis, margine magis perspicue diaphanis, 2–4 mm. longis. Achaenia linearia, plana vel subtetragona, fusco-atra, supra faciebus marginibusque erecto-hispida, corpore 3–5 mm. longa et 0.3–0.9 mm. lata,

apice biaristata aristis adscendentibus, tenuibus, erecto-scabridis, 1–2 mm. longis.

Type specimen: *George Gardner* 4253, near Senhora d' Abbadia, State of Goyaz, Brazil, in 1841 (Herb. Kew).

Distribution: States of Goyaz and Sao Paulo, Brazil.

Specimens examined: *G. Gardner* 4253 (Herb. Kew, type; Herb. Gray); *A. Glaziou* 21557, Cassú, Goyaz, August 5, 1894 (Herb. Berl.); *A. T. Regnell* ser. III. 738 (per errorem *Baker* 783 dixit), Sao Paulo, February, 1847 (Herb. Berl.); *anon.* (verisimiliter *Riedel*) 98, Paraná River, Brazil (Herb. Deless.) *L. Riedel* 410, in saxosis fissuris rupium ripae fluminis Paraná, prope Urubupunga, Brazil (Herb. Gray; Herb. Kew).

BAKER distinguished his *Isostigma stellatum* from *I. brasiliense* by means of the leaves, referring to the former those with simple leaves and to the latter those with tripartite leaves. Incidentally, he described the peduncles for the former as 5–10 cm. ("2–4 poll.") long, and for the latter as 15–22.5 cm. ("6–9 poll.") long. These distinctions are seen not to hold among further specimens examined by me. Thus, while *Riedel* 410 (*ex* Herb. Petrop.) at Kew has the leaves all simple, the anonymous 98 (*ex* Herb. Petrop. in Herb. DC.) at Geneva, a specimen which probably also was collected by RIEDEL, has the leaves slightly longer, namely, 4–7 cm. long, and the many leaves of both simple and tripartite types. Of the more recent Glaziou specimens (five on one sheet), two have mainly tripartite leaves and three have also great numbers of simple leaves, in some cases the leaf clusters being composed exclusively of a score or more of simple leaves. Furthermore, the Glaziou plants, as also the REGNELL plant at Berlin (of which BAKER cited a duplicate for *I. brasiliense*), have short peduncles as in BAKER's material of *S. stellatum.* Thus the length of the peduncles as well as the nature of the leaves is seen to be a fickle character. An examination of other characters fails to show any grounds for treating the several forms as other than members of one species.

2. ISOSTIGMA ACAULE (Baker) Chod., Bull. Herb. Boiss. ser. II. 1:417. 1901; *Bidens acaulis* Baker, Martius Fl. Brasil. 6^{III}:247. 1884; *Isostigma Vailiana* Britt. in MORONG and BRITTON Enum. 149. 1892.—Pl. XXIII, figs. *a*, *b*.—Herba 1.5–2.5 dm. alta, caulibus basi decumbentibus. Folia plura, rosulata, subpetiolata, rigidula, cuneato-oblanceolata, apice plerumque 3 (interdum–7)-dentata, 1.5–7 cm. longa et 4–12 mm. lata. Capitula solitaria caules elongatos terminantia, discoidea, demum circ. 1 cm. alta et 1.5 cm. lata. Involucrum campanulatum, bracteis adpressis, rigidulis, anguste lanceolatis, acutis, viridulis margine pallidis, subaequilongis (5–7 mm.). Flores disci saturate purpureo-rubri, 4-dentati. Achaenia linearia, sub-

tetragona, hispidula, corpore 8–10 mm. longa et circ. 1 mm. crassa, biaristata aristis scabris 2–3 mm. longis.

Type specimen: *B. Balansa* 913, in clay soil, Plain of Arégua, Paraguay, January, 1875 (Herb. Kew).

Distribution: Paraguay.

Specimens examined: *B. Balansa* 913 (type in Herb. Kew; cotype, Herb. Hort. Petrop.); *Dr. Emil Hassler* 1030, in field near Tacuaral, Paraguay, September, 1885–95 (Herb. Hassl. in Herb. Deless.); *idem* 3811, in field at Tacuaral, Paraguay, January, 1899 (Herb. Hassl. in Herb. Deless.); *Thomas Morong* 734, Limpio, central Paraguay, May, 1888–1890 (Herb. Gray; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat.).

A species with the general aspect of *Glossogyne tridentata* (Turcz.) B. and H. *ex* Ind. Kew. of the Philippine Islands, but growing considerably larger.

3. **Isostigma scorzoneraefolium** (Baker), comb. nov.; *Bidens scorzoneraefolia* Baker, MARTIUS Fl. Brasil. 6^{III}:247. 1884; *Isostigma glycinaefolium* (Schz. Bip. *ex* Baker) SHERFF, BOT. GAZ. **76**:78. *pl. 6.* 1923; *Bidens glycinaefolia* Schz. Bip. *ex Baker, l.c.; Isostigma foliosum* Malme, Svensk. Vet. Akad. Handl. **32**^{V}:66. *pl. 6. fig. 17.* 1899.—Pl. XXIII, figs. *g, h.*—Herbacea, 5–7 dm. alta; caule simplici nisi versus apicem, subtereti, inconspicue angulato, supra sparsim folioso vel subnudo, infra dense folioso, e basi lignosa. Folia glaucescentia, eis graminearum valde similia, sessilia, inferiora 8–19 cm. longa et 4–10 mm. lata, conferta, superiora sensim minora et distantia, omnia alterna vel perpauca opposita, indivisa, linearia, elongata, apice acuminata, infra angustata, basi ipsa plerumque dilatata atque amplexicaulia, integra vel parce antico-serrata dentibus angustis acribusque, rigidula et parce membranacea. Capitula pauca (circ. 4–9), laxe corymbosa, ramulos 3–14 cm. longos coronantia, radiata, pansa ad anthesin 2–2.5 cm. lata et fere vel etiam 2.5 cm. alta. Flores ligulati 8–14, flavi luteive, ligula lineares, apice acriter bidentati, 9–13 mm. longi; paleis versus apicem perspicue longo-attenuatis, achaeniorum corpora superantia; disci floribus ad apicem acriter 4-dentatis; disco demum circ. 12 mm. lato et 6–9 mm. alto. Involucrum glabratum vel minutissime pubescenti-scabridum tuberculo-rugulosumve, bracteis plus minusve 3-seriatis, rigidulis, adpressis, lanceolatis, margine saepe erosis et etiam ciliatis, extimis 3–7, acutis, 3–5 mm. longis; interioribus lanceolatis, ad apicem saepe attenuatis, paulo longioribus. Achaenia linearia vel anguste lineari-

cuneata, plana vel obcompresso-tetragona, fusco-atra vel rubronigrescentia, exalata, marginibus ac costis medianis suberecte setosa, alibi glabra, corpore 5–8 mm. longa et circ. 1 mm. lata, biaristata; aristis tenuibus, erectis, calvis vel versus basim arrecte setulosis (vel omnino rigide ciliatis ex Baker), 1–2 mm. longis.

Type specimen: *Patricio Da Silva Manso* 215, at Cuyabá, State of Mattogrosso, Brazil, 1834 (Herb.[3] Bruss.).

Distribution: Vicinity of Cuyabá, State of Mattogrosso, Brazil.

Specimens examined: *Gust. Malme* 1584, in sandy-gravelly, grassy, subhumid, open field near Coxipó (igreja), vicinity of Cuyabá, April 26, 1894 (cotypes of *I. foliosum* Malme; Herb. Berl.; Herb. Brit. Mus.); *Patricio Da Silva Manso* 215, Cuyabá, 1834 (type in Herb. Bruss.); *R. Pilger* 479, scattered upon stony ground at foot of Serra das Pedras in Cuyabá Valley, April 15, 1899 (Herb. Berl., two sheets); *L. Riedel*, in subhumid, grassy places, near Cuyabá, May, 1827 (Herb. Kew; Herb. Par.; Herb. Petrop.).

BAKER described his *Bidens scorzoneraefolia* from the upper portion of MANSO's plant, collected at Cuyabá. Had BAKER seen an entire plant with its interesting basal leaves, or had he at least known that the type of *B. glycinaefolia*[4] was collected at the same station, he doubtless would have been led to compare the two. In any case, BAKER's description shows that he was dealing with the same species which he described, further down on the same page, under the name *Bidens glycinaefolia* Schz. Bip.

MALME's *Isostigma foliosum*, unknown to me until recently, is seen from its cotypes and type illustration to be synonymous with this species, and, moreover, to have been collected in *I. scorzoneraefolium's* type locality, Cuyabá.

4. ISOSTIGMA HERZOGII Hassl., Fedde Rep. Spec. Nov. **7**:358. 1909.—Pl. XXIV, figs. *i, o, r, t, u*.—Herba 5–6 dm. alta, caule simplici ramulis simplicibus apice floriferis paucis aucto, tereti, manifeste et prominenter striato, ad basim circ. 3 mm. crasso. Folia basalia verisimiliter more generis rosulata (in typi specimine unico incompleto folium basale unicum adest); caulina vulgo alterna, rarius plus minusve opposita, distantia internodiis 2–5 cm. longis, basali similia sed minora, omnia lineari- vel oblongo-lanceolata, basi sessilia

[3] BAKER did not cite the herbarium possessing the type, but recently, through the aid of Dr. KARL GOEBEL of Munich, I was able to locate it among the MANSO plants sent to VON MARTIUS and deposited now in Brussels. I am indebted to Dr. E. DE WILDEMAN for his having allowed me to make an examination of the specimen.

For interesting information as to the type locality, Cuyabá, the reader is referred to S. L. MOORE, Trans. Linn. Soc. 2d Ser., Bot. 4:265–270 *et sequ.* 1895.

[4] For an extended discussion of the type of *Bidens glycinaefolia*, see SHERFF, BOT. GAZ. **76**:78. 1923.

semiamplexicaulia, apice acuta, subcoriacea, concoloria, margine eburneo cincta basale ±6.5 cm. longum et ±6 mm. latum, caulina 2–4 mm. lata. Capitula ad apices caulis et ramulorum in corymbis 3–4-capitulis, odore (fide Herzog) Nigritellae similia, satis longe pedicellata, pedicellae bracteis 1–3 foliis caulinis similibus sed minoribus. Involucrum urceolato-campanulato, bracteis exterioribus anguste triangularibus, crassiusculis, leviter reflexis, margine albicanti subhyalino lacerato-fimbriato cinctis, 3 mm. longis et 0.75 mm. latis; interioribus oblongis vel ovato-oblongis, tenuioribus, margine latiusculo ei bractearum exteriorum simili cinctis, 5–6 mm. longis et 1.2–2 mm. latis. Flores ligulati atro-purpurei, ligula 9–11 mm. longi, stigmatibus longe appendiculatis, aequilongis, ovario oblongo sparse setuloso, 3 mm. longo et 1 mm. lato, aristis subulatis, patulis 1.5–2 mm. longis; paleis linearibus, apice acutis, 8–9 mm. longis et vix 0.2 mm. latis. Achaenia oblonga, versus basim angustata, plana vel obcompressa, flava, medio superiore setulis sparsis hispidula, 12 mm. longa et 1 mm. lata, aristis subulatis 2 mm. longis.

Type specimen: *Dr. Theodor Herzog* 617, abundant in field, alt. about 600 m.. Santiago de Chiquitos, Dept. of Santa Cruz, Bolivia, May, 1907 (Herb. Hassl. in Herb. Deless.).

Distribution: Known only from type locality in southeastern Bolivia.

Specimens examined: *Herzog* 617 (type).

The tiny fragment studied by HASSLER was much too scanty to afford a broad and adequate idea of the species as a whole. It was only about 1.5 dm. long. The species would seem, however, to be like *I. scorzoneraefolium* in habit, but like *I. Hoffmannii* in color of its rays.

5. ISOSTIGMA SIMPLICIFOLIUM Less., Linnaea **6**:513. 1831.—Pl. XXIII, figs. *c, d, e, f, v, w*.—Herba erecta, 3–6 dm. alta, caulibus paucis e rhizomate crasso lignoso, simplicibus. Folia ad basim pedunculi longi dense rosulata, alterna, simplicia, sublinearia, basi longe angustata, coriacea, nervis parallelis multis striata, apice acuto vel obtusiusculo plus (usque ad 5-) minusve denticulata, nonnumquam obsolete trifida, plus minusve curvata, nitida, 7–13 (vel etiam–25) cm. longa et 1.5–5 mm. lata. Capitula solitaria, ligulata, pedunculis 1–2 (vel etiam usque ad 6)-squamatis, florescentia (ligulis non inclusis) circ. 1.5–2 cm. latis et circ. 1.3 cm. alta. Involucrum campanulatum, biseriale, glabrum, bracteis exterioribus circ. 8–10, linearibus, acutis, circ. 2.5–4.5 mm. longis, interioribus

lanceolatis margine scariosis, apice subobtusis et irregulariter plus minusve dentatis, circ. 10–12 mm. longis. Flores ligulati magis minusve profunde 3-partiti, atro-purpurei, disco subaequantes; disci atro-purpurei. Achaenia linearia, nitida, incurva, striata, circ. 12 mm. longa et 2 mm. lata, anguste alata, alis integerrimis in aristulas binas, tenues, laevissimas, latitudinem ovarii aequantes excurrentibus.

Type specimen: *Sello* 873 (numbered also 1111), Facienda da Porteira, in 1818 (Herb. Berl.).[5]

Distribution: Eastern Brazil.

Specimens examined: *Claussen*, in fields at Cachoeira do Campo, Minas Geraes, Brazil (Herb. Kew); *Sello* 873 and 1111 (3 type sheets, Herb. Berl.).

6. Isostigma Hoffmannii O. Kuntze Rev. Gen. 3^{II}:160. 1898.—Pl. XXIV, figs. *h*, *p*, *q*, *s*.—Herba suffrutex, parvus, e rhizomate lignoso, tantum 1–4 dm. alta, caulibus simplicibus, infra plerumque arcuatis, supra nudis vel remotissime 1–3-squamatis. Folia nunc ad basim caulis dense rosulati, nunc per caulem inferiorem opposite alterneve disposita, petiolata petiolis subangustis vel alatis usque ad 2.5 cm. longis et ad basim saepe connatis, petiolo adjecto 1.5–5.5 cm. longa, plus minusve bipinnati- vel biternatisecta, costa mediana segmentisque linearibus, planis, 0.5–3 mm. latis, apice acutis vel etiam acuminatissimis. Capitula solitaria, moderate parva, circ. 50–60-flora, longe pedunculata, radiata, pansa ad anthesin 2.5–3.5 cm. lata et 1–2 cm. alta. Involucrum campanulatum, bracteis irregulariter 2–4-seriatis, adpressis, exterioribus linearibus, viridibus, apice acutis, margine ciliatis, 3–5 mm. longis, intimis lanceolatis, plerumque 4–6 mm. longis. Flores ligulati, parvi, fusco-rubri vel luteo-rubri, ligula lineares, apice bidentati, 6–9 mm. longi; tubulosi apice 4-dentati. Achaenia linearia, obcompressa, atro-fusca, marginibus et nervis et apice erecto-setosa, margine anguste alata, cor-

[5] One of the three sheets in Berlin has the locality, Facienda da Porteira. A second gives merely Brasilia. The third bears two specimens (*a Humboldt ex reliquiis Selloianis anno 1836 datis*). One of these was correctly named *I. simplicifolium* and has a label similar to that for the first (Facienda da Porteira, etc.). The other was unnamed and without a number, but is *I. crithmifolium*, and the locality is S. Antonio do Monte. It is perhaps from this last plant that Baker obtained the locality S. Antonio do Monte for *I. simplicifolium*, a species to which he may have referred the plant of *I. crithmifolium*. Baker (Martius Fl. Brasil. 6^{III}:240. 1884) cited also S. Antonio do Monte for *Sello's* 873 and 1111.

pore 6–10 mm. longa et circ. 1 mm. lata, perspicue biaristata aristis tenuibus, divergentibus, omnino laevissimis vel versus basim erecto-barbellatis, 3.5–5 mm. longis.

Type specimen: *Dr. Otto Kuntze,* alt. 400 m., Rio Yapacani, Dept. of Santa Cruz, Bolivia, June, 1892 (Herb. Berl., 2 sheets).

Distribution: Southern Bolivia and northern Argentina.

Specimens examined: *Alcide D' Orbigny* 639, Dept. of Santa Cruz, Bolivia, May, 1826–1834 (Herb. Par.); *Th. Herzog* 234, alt. about 400 m., abundant on the savanna pampas of Santa Cruz de la Sierra, Dept. Santa Cruz, Bolivia, November, 1907 (Herb. Berl.); *Dr. Otto Kuntze*, Rio Yapacani, Dept. Santa Cruz, Bolivia (2 type sheets, Herb. Berl.; Herb. U.S. Nat.); *P. G. Lorentz*, Prov. of Santiago del Estero, Argentina, February, 1874 (Herb. Berl., *sub nom. I. Lorentzii* Hieron.); *H. Alg. Weddell* 3569, Santa Cruz de la Sierra, Dept. Santa Cruz, Bolivia, November, 1845 (Herb. Par.).

KUNTZE (*l.c.*) described the disk florets as 5-dentate at the top, but I have found only 4-dentate florets in his type material. HIERONYMUS (in Herb. Berl.) had remarked upon this same 4-dentate character in the LORENTZ material, and had given LORENTZ'S plant the name (apparently heretofore unpublished) *Isostigma Lorentzii*.

7. ISOSTIGMA CRITHMIFOLIUM Less., Linnaea 6:515. 1831; BAKER, MARTIUS Fl. Brasil. 6^{III}:241. *pl. 71. fig. 1.* 1884.—Pl. XXIV, figs. *b*, *c*.—Herba erecta, 5–7 dm. alta, caulibus caespitosis, simplicibus, tenuibus, remotissime squamulatis. Folia ad basim caulis (pedunculi) dense rosulata, petiolo adjecto 1.2–2.5 dm. longa, in dimidio superiore 5–7-furcata, foliolis linearibus, inferioribus rursus furcatis, segmentis planis, 0.5–1.3 mm. latis, ultimis segmentis 2–6 cm. longis. Capitula solitaria, moderate magna, radiata, pansa ad anthesin circ. 5 cm. lata et 1.8–2.3 cm. alta, sub basi solum 1–2 squamulis praedita. Involucri campanulati bracteae exteriores 7–10, virides, adpressae, lineares, plerumque e basi usque ad apicem acutum sensim angustatae, 3–6 mm. longae; interiores lanceolatae, margine scariosae, 10–15 mm. longae. Flores ligulati circ. 12–15, extra brunneo-purpurei intus plerumque albido-argentei lineares, apice plerumque 3-dentatae, 1.8–2.5 cm. longi. Achaenia (fide Baker) linearia, 1.6–1.8 cm. longa, aristis minutis patulis lanceolatis.

Type specimen: *Sello*,[6] Uruguay, in 1823 (Herb. Berl.).

[6] Lessing cited no particular number of SELLO'S. The Berlin Herbarium contains four sheets of SELLO material from *Brasilia* (as then bounded). Two sheets are labeled "Salto, Febr., 23" and one is labeled "S. José del Uruguay, Feb., 23," whence it is seen that SELLO collected the types in what is now Uruguay.

Distribution: Uruguay and Province of Entre Rios, Argentina.

Specimens examined: *Dr. P. G. Lorentz* 862, in pastures, between Yucari grande and Chico, Province of Entre Rios, Argentina, February 20, 1876 (Herb. Berl.); *Sello*, Uruguay (4 sheets of type material, Herb. Berl.).

8. Isostigma dissitifolium Baker, Martius Fl. Brasil. 6^{III}: 239. 1884; *I. peucedanifolium* var. *dissitifolium* (Baker) Hassler, Fedde Rep. Spec. Nov. 14:277. 1916.—Pl. XXIV, figs. *a*, *f*, *j*.—Herba erecta, caulibus multis caespitosis parce ramosis, 0.6–1 m. altis. Folia caulina plura segregata, opposita vel alterna, petiolata petiolis tenuibus 1–3 cm. longis, petiolo adjecto 5–10 cm. longa, 5–7-furcata, segmentis lineari-subulatis 0.5–1 mm. latis, inferioribus furcatis. Capitula solitaria, radiata, pansa ad anthesin 4–5 cm. lata et 1–2 cm. alta, longissime pedunculata pedunculis usque ad 4 dm. longis, foliis 1 vel 2 minutis squamosis praeditis. Involucrum campanulatum, bracteis exterioribus circ. 8–10, anguste linearibus, viridulis, adpressis, 3–10 mm. longis; interioribus oblongo-lanceolatis, rigidulis, griseo-violaceis vel brunneo-purpurascentibus, 8–13 mm. longis. Flores ligulati ± 10, extra rubro-brunnei intus plerumque albido-argentei, linearia, apice profunde acerrimeque dentati, 1.5–2 cm. longi. Achaenia linearia, obcompressa, faciebus subatra, marginibus subalatis straminea, omnino glabra, corpore 1.1–1.7 mm. longa et 1.5–2 mm. lata, apice biaristata aristis patulis erectisve tantum usque ad 1 mm. longis.

Type specimen: *B. Balansa* 907, on uncultivated hills, Guarapi, district of Yaguaron, Paraguay, June, 1877 (Herb. Kew).

Distribution: Paraguay.

Specimens examined: *B. Balansa* 907 (type in Herb. Kew); *K. Fiebrig* 450, high, rocky places, among grasses, Cordillera de Altos, Paraguay, November, 1902 (*sub nom. Isostigma Fiebrigii* Hieronymus, Herb. Berl., 3 sheets; Herb. Deless., 2 sheets; Herb. Field Mus.; Herb. Gray, 2 sheets; Herb. Hassl. in Herb. Deless.; Herb. U.S. Nat., 2 sheets); *Dr. E. Hassler* 1074, in field near Itacurubi, Paraguay, September, 1885–95 (Herb. Hassl. in Herb. Deless.); *idem* 1509, sandy fields, San Bernardino, Paraguay, October, 1915 (Herb. Hassl. in Herb. Deless.); *idem* 3944, Paraguay (Herb. Hassl. in Herb. Deless.); *idem* 6309, in region of hills, "Cerros de Tobaty," central Cordillera, Paraguay, September, 1900 (Herb. Berl.; Herb. Gray; Herb. Hassl. in Herb. Deless.).

Fiebrig (*in herb.*) states that the leaves when rubbed smell like celery. Hassler (*l.c.*) states that this species differs from *I. peucedanifolium* (Spreng.) Less. (=*I. megapotamicum*) only in having the cauline leaves large and unre-

duced, but the matter of leaf reduction and leaf distribution in *Isostigma* seems a fairly important criterion. I have found no transitions connecting the two forms.

9. **Isostigma megapotamicum** (Spreng.), comb. nov.; *Bidens megapotamica* Spreng. Syst. Veg. **3**:454. 1826; *Tragoceras peucedanifolium* Spreng. (*fide* Less.) *l.c.* 576; *Isostigma peucedanifolium* (Spreng.) Less., Linnaea **6**:514. *pl. 6. figs. 1–5.* 1831.[7]—Pl. XXIV, figs. *g, k, l, m, n, v.*—Herba erecta, 5–10 dm. alta, e rhizomate lignoso, caulibus simplicibus, caespitosis, squamis 1–3 minutis, subulatis, obsitis. Folia ad basim caulis tenuis (pedunculi) dense rosulata, 1.2–3.3 dm. longa (petiolo adjecto), in dimidio superiore 5–7-furcata, segmentis adscendentibus et petiolis similibus, inferioribus plerumque furcatis, ultimis tenuissime linearibus, trigonis, supra canaliculatis, 3–12 cm. longis et tantum 0.2–0.7 mm. latis. Capitula solitaria, plerumque radiata, saepe speciosa, pansa ad anthesin 3–5 cm. lata et 1.7–2 cm. alta. Involucri campanulati bracteae glabrae exteriores 8–16, lineares vel flagelliformes, supra attenuatae, apice acutae, nunc tantum 5–7 nunc etiam usque ad 28 mm. longae, interiores lanceolatae usque ad 2 cm. longae. Flores ligulati circ. 15, extra atro-purpurei intus plerumque albido-argentei, ligula lineares, apice profunde 2–3-dentati, 1.8–2.5 cm. longi; paleis rigidulis, brunneo-purpureis, lineari-lanceolatis, 12–15 mm. longis, quam achaeniis nunc brevioribus nunc longioribus. Achaenia linearia, obcompressa, brunnea vel atro-brunnea, nitida, glabra, striata, 12–18 mm. longa et 1.2–2 mm. lata, anguste alata, apice aristulis binis, marginalibus, laevissimis, patenti-divergentibus, 0.5–2 (rarius usque ad 4.5) mm. longis, interdum deciduis, plerumque coronata.

Type specimen: Collected by SELLO, probably in the State of Rio Grande do Sul, Brazil,[8] but perhaps in Uruguay (no herbarium cited by SPRENGEL, but see later discussion).

Distribution: Eastern and southern Brazil; Paraguay; perhaps also in adjacent portion of Argentina and in Uruguay.

Specimens examined: *Alfred Bornmüller* 761, New Wurttemberg, Rio Grande do Sul, Brazil, December 7, 1906 (Herb. Berl.); *Burchell* 5259, tropical

[7] For *I. peucedanifolium* var. *genuinum* Hassl. *cum* f. *discoideo* Hassl. *et* f. *radiato* Hassl., see under *L. speciosum* Less.

[8] For geographic note in this connection see SHERFF, BOT. GAZ. **76**:92 (footnote 11). 1923.

Brazil (Herb. Gray); *P. Dusen* 3228, in field, Paraná, Brazil, December 14, 1903 (Herb. Berl.); *Dr. A. Glaziou* 20381, Minas Geraes, Brazil, 1893–94 (Herb. Berl.; Herb. Kew); *Dr. E. Hassler* 10366, in dry field, Punta Para, Sierra de Amambay, Paraguay, April, 1908 (Herb. Hassl. in Herb. Deless.); *Gust. A. Malme* 1158, dry, open, sandy-gravelly place, Cuyabá, Mattogrosso, Brazil, November 24, 1893 (Herb. Berl.); *G. Niederlein*, between Corrientes, Argentina, and Santa Ana, Paraguay, January 22, 1883 (Herb. Berl.); *Capt. Page* (La Plata Exped.), Paraná, Brazil (Herb. U.S. Nat., a form with aristae up to 1.5 mm. long); *R. Pilger* 493, upon stony ground at foot of the Serra das Pedras, in Cuyabá Valley, Mattogrosso, Brazil, April 15, 1899 (Herb. Berl.); *A. F. Regnell* ser. III. 782, Uberava, Minas Geraes, Brazil, September 29, 1848 (Herb. Berl.; Herb. U.S. Nat.); *E. M. Reineck* and *Joseph Czermak* 273, sunny slopes of the Parthenon Mountains, Porto Alegre, Rio Grande do Sul, Brazil, December, 1898—January, 1899 (Herb. Berl.; Herb. Deless., 2 sheets); *L. Riedel*, Brazil (Herb. Gray, 2 sheets); *idem* 797, Brazil (Herb. Gray; the slender aristae measuring 1–2 mm. long); *Sello* 1723 *pro parte*, 3327 and 5179 (8 sheets of type material, Herb. Berl.; 3327 also in Herb. Gray); *idem*. Brazil (Herb. Gray; Herb. Kew, 2 sheets); *Tweedie* 165, Rio Jacuhy, Rio Grande do Sul, Brazil, in 1837 (Herb. Kew).

With original, authentically named specimens of *Bidens megapotamica* Spreng., LESSING evidently was not familiar. In his *Synopsis Compositarum*, published (1832) six years after SPRENGEL's description, he completely omits this name. DE CANDOLLE (Prodr. **5**:604 1836) had seen no authentic material to represent it and listed it as one of his "*Species non satis notae*." I have already (BOT. GAZ. **76**:92. 1923) shown that HOOKER and ARNOTT (HOOKER etc. Jour. Bot. **3**:319. 1841) equated it with *Thelesperma scabiosoides* Less. This equation was given again by BAKER (Mart. Fl. Brasil. 6^{III}:249. 1884), and in 1898 OTTO KUNTZE, evidently following BAKER's treatment, created the name *Thelesperma megapotamicum* for *T. scabiosoides* Less. The Berlin Herbarium contains four SELLO sheets of *Thelesperma scabiosoides* Less. While these were assumed by me in a former paper (*l.c.*), in view of KUNTZE, BAKER, and HOOKER and ARNOTT's treatments, to have been part of the original collection of *Bidens megapotamica* Spreng., it must be noted that none of them bears the name *B. megapotamica* Spreng. This suspicious fact led me in 1924 to a careful search among the Berlin Herbarium specimens, and later through those at the British Museum of Natural History and at Kew. In none of these places was a SELLO specimen found with the name *Bidens megapotamica* Spreng. accompanying it. Recently, however, through the great kindness of Dr. JOHN BRIQUET, in charge of the Delessert Herbarium in Geneva, I have been lent many hundreds of additional sheets of material. Among these is a specimen such as I had hoped to find at Kew. It bears the label "*Brasilia Sello*," and on the sheet, in badly faded writing, says "*Bidens megapotamica* Spreng." It is mounted on ribbed paper (water-marked "*J. Whatman* 1829," the paper and style of mounting being

in the regulation Kew style of earlier years). It originally belonged to Lambert's Herbarium, whence it was purchased by DELESSERT (*fide* Briquet *in litt.*), and so may have been seen, although misinterpreted, by HOOKER and ARNOTT in England. It is the *Isostigma peucedanifolium* of Lessing. Its heads are somewhat past the flowering stage, and, having lost their rays, appear discoid. SPRENGEL'S description is seen to apply to this plant much better than to plants of *Thelesperma scabiosoides* Less.: "B. foliis omnibus 2-pinnatifidis lineari-filiformibus glabris, floribus subgeminis pedunculatis erectis discoideis, involucro colorato anthodium aequante." The term "floribus subgeminis" apparently is based upon the tendency of the full-sized heads to sag somewhat on both sides of the peduncle, when pressed flat upon the sheet, and to flare outward at the top as if possessed with a tendency to become more or less cleft or double later on.[9]

10. ISOSTIGMA RIEDELII Schz. Bip. *ex* Baker in MARTIUS Fl. Brasil. 6^{III}: 240. 1884; also in Ind. Kew. 2: 1240. 1893; *cf.* CHODAT, Bull. Herb. Boiss. ser. II. 2: 394. 1902; *ibid.* 3: 725. 1903.[10] *I. speciosum* var. *Riedelii* (Schz. Bip. *ex* Bak.) Baker, *l.c.*—Pl. XXIII, figs. *i, j, k, l, m, u, x, y.*—Herba erecta, virgata, 8–13 dm. alta, caulibus e rhizomate lignoso interdum etiam 5 cm. crasso ortis. Folia principalia numerosa, ad basim caulis subnudi (pro pedunculo) confertissima, maxime elongata, pallida, nervis parallelis perspicue striata

9 I have seen no SELLO sheets with the original label of *Tragoceras peucedanifolium* Spreng. SPRENGEL'S description was meager and unsatisfactory. He cited no herbarium, nor did LESSING. Yet LESSING'S retention of SPRENGEL'S trivial name *peucedanifolium* shifts LESSING'S type basis back, of course, to whatever SELLO plant SPRENGEL may have studied. In this case, it is doubly reassuring to trace the species back to the first trivial name *megapotamicum* and find an undoubtedly authentic plant to represent it.

10 BAKER (*l.c.*) says, "Var. *B. Riedelii* (Schultz Bip. *in sched.* Riedel, *s. sp.*)." The *Index Kewensis* published the name more definitely as "*Isostigma Riedelii* Schz. Bip. *ex* Baker," in harmony with its general procedure in somewhat comparable cases. Thus, as an example, for *Glossogyne brasiliensis* Gardn., BENTHAM and HOOKER (Gen. Pl. 2: 389. 1876) merely stated that it should go to *Isostigma*. Whereupon the *Index Kewensis* published the name suggested, but not printed, by BENTHAM and HOOKER, namely, *Isostigma brasiliense* (Gardn.) B. and H. (It is significant, however, of the *Index Kewensis* authors' own misgivings as to the absolute validity of such a step that, in the corresponding case of *Bidens purpurea* DC., listed previously to the *Index Kewensis* as *Cosmos purpureus* [DC.] B. and H. by HEMSLEY [Biol. Centr. Amer. Bot. 2: 200. 1881], the *Index Kewensis* credited the name's publication not to "BENTHAM and HOOKER," but to "BENTHAM and HOOKER . . . . *ex* HEMSLEY"). CHODAT (*l.c.*) assumed credit for making the specific name *I. Riedelii*, and several botanists have followed him. But clearly, regardless of the merits of its procedure, the *Index Kewensis* must be regarded as having removed any technical shortcomings in connection with the name *I. Riedelii*, and as having given it effective publication years before CHODAT'S treatment.

simplicia, integra vel apice saepe plus minusve profunde trifida, 2.5–4 dm. longa, ultima parte (dimidia-quarta) curvata, linearia et infra sensim angustata, plerumque 4–6.5 mm. lata, residua parte (pro petiolo) angustiora (1–2 mm. lata) et crassiora. Capitula magna, speciosa, solitaria, radiata, pansa ad anthesin 5–6 cm. lata et 1.7–2 cm. alta, pedunculis fere usque ad basim caulium decurrentibus, foliis reductis multis (saepe sub capitulo perspicue numerosis) per omnem partem praeditis. Involucrum campanulatum, bracteis tergo minute glanduloso-scabridis, exterioribus circ. 25 imperfecte biserialibus, linearibus, apice acutis, margine obsolete ciliato saepe albis, usque ad 13 mm. longis, interioribus lanceolatis, margine scariosis, saepe brunneo-purpurascentibus, 1.2–1.5 cm. longis. Flores purpurascentes; ligulati plerumque circ. 12–15 (rarius usque ad 30–32) ligula lineares, intus plus minusve albido-argentei, apice minute sed acriter 2 vel 3 dentulis denticulati, 2–2.5 cm. longi; paleis anguste linearibus, ad apicem purpureis, achaenia matura perspicue superantibus. Achaenia late linearia, obcompressa, striata, glaberrima, nitida, corpore 1.3–1.5 cm. longa et 2–2.5 mm. lata, faciebus atra vel flavido-atra, marginibus anguste alata, alis integerrimis, flavidis, in aristulas binas laevissimas, obscuras vel usque ad 1.5 mm. longas excurrentibus.

Type specimen: *L. Riedel*, in dry fields along the banks of the Rio Pardo, Brazil (Herb. Kew).

Distribution: Paraguay and southern Brazil.

Specimens examined: *K. Fiebrig* 4755, among bushes, dry field at edge of forest, Villa Lana, between the Rio Apa and the Rio Aquidaban, northern Paraguay, January, 1909 (Herb. Berl.; Herb. Deless.; Herb. Gray); *Dr. E. Hassler* 567, fields, Serra dos Esperanza, Sierra de Amambay, Paraguay (Herb. Hassl. in Herb. Deless.); *idem* 5978, on high plateau near the Rio Tapiraguay, Paraguay, December, 1898 (Herb. Hassl. in Herb. Deless.); *idem* 8047, in region of the upper part of the Rio Apa, northern Paraguay, November, 1901 (Herb. Berl.; Herb. Gray; Herb. Kew); *idem* 9238, in the vicinity of Caaguazú, Paraguay, March, 1905 (Herb. Berl.); *L. Riedel*, Brazil (Herb. Kew, type; Herb. Gray); *T. Rojas* (*Dr. E. Hassler distrib.*) 9927, on plateau and slopes, "Sierra de Amambay," Paraguay, December, 1907 (Herb. Berl.); *idem* 10567, *eodem loco*, August, 1907 (Herb. Berl.).

A species with foliar habit much like that of *I. simplicifolium*, although the leaves probably average longer and perhaps are more furcate at the apex. The robust peduncle with its many bracts near and at the apex, also the normal (not dwarfed) rays indicate, however, a close affinity with *I. speciosum*.

11. ISOSTIGMA SPECIOSUM Less., Linnaea **6**:515. 1831; *I. peucedanifolium* var. *genuinum* f. *discoideum* Hassl. *et* f. *radiatum* Hassl., Fedde Rep. Spec. Nov. **14**:277. 1916.[11]—Pl. XXIV, figs. *d, e*.—Herba erecta, e rhizomate lignoso, 6–10 vel etiam (fide Hassl. in herb.) usque ad 25 dm. alta, caulibus simplicibus, caespitosis, apice incrassatis et valde squamosis, squamulis linearibus, acutis vel subobtusis, in foliola involucri transeuntibus. Folia ad basim caulis (pedunculi) dense rosulata, petiolata petiolis plerumque usque ad 2 (rariter usque ad 2.7) dm. longis, petiolo adjecto 1.5–3.5 dm. longa, crassa, profunde 3–4-furcata, segmentis petiolo similibus, adscendentibus, subulatis, rigidis, saepe trigona apice acutis, 5–13 cm. longis et 0.9–1.2 mm. latis. Capitula solitaria, magna speciosaque, radiata, pansa ad anthesin 4.5–5.5 cm. lata et 1.5–2 cm. alta, demum cum paleis achaeniisque maturis circ. 2.3 cm. alta. Involucri campanulati bracteae exteriores e caulis superioris (pro pedunculo) squamulis non acriter distinctae, lineares, acutae, adpressae, nunc omnino viridulae et usque ad 1.3 cm. longae, nunc plus minusve albo-marginatae et in bracteas interiores lanceolatas, lateraliter diaphanas, usque ad 2 cm. longas transeuntes. Flores (ex Fiebrig odore melli similes) ligulati circ. 15, extra atro-purpurei intus albido-argentei, ligula lineares, apice plus minusve 3-dentati, 2–2.5 cm. longi; paleis linearibus, achaenia matura facile superantibus. Achaenia oblongo-linearia, obcompresssa, atro-fusca, glabra, corpore circ. 1.6–1.8 cm. longa et circ. 2 mm. lata, anguste alata alis in aristas binas, patulas, laevissimas, obsoletas vel usque ad 1.2 mm. longas excurrentibus.

Type specimen: *Sello*, Brazil[12] (Herb. Berl.).

Distribution: Paraguay and southern Brazil.

Specimens examined: *P. Dusen* 4345, in field, in vicinity of Ponta Grossa,

[11] HASSLER's var. *genuinum* itself, described (*l.c.*) "e specimine typico Herb. Prodromi," doubtless must be referred to *I. speciosum*. His description ("pedunculis foliis reductis linearibus ad 1, 5 cm. longis 1/4 mm. latis alternis-±numerosis ornatis. . . . .") characterizes *I. speciosum* very well.

[12] LESSING (*l.c.*) cited no particular number of SELLO's. I have before me the six sheets of SELLO's original material belonging to the Berlin Herbarium. There are three sheets without number and, of the remaining three, one each bears SELLO's number 243, 1112, and 1723. All six lack the locality, other than Brasilia. The sheet with no. 1723 bears primarily *I. megapotamicum* and has one specimen of *I. speciosum* merely through careless admixture.

SHERFF on ISOSTIGMA

SHERFF on ISOSTIGMA

Paraná, Brazil, March 7, 1904 (Herb. Berl.);[13] *Sello* 243, 1112, 1723 *et sine num.* (type material, 6 sheets in Herb. Berl.).

HASSLER based his *I. peucedanifolium* var. *genuinum* f. *discoideum* upon *Fiebrig* 810, in Herb. Hassl. The five specimens of *Fiebrig* 810 examined by me are in no way to be distinguished from ordinary *I. speciosum*. Several of the heads are well developed and have normal rays. His *I. peucedanifolium* var. *genuinum* f. *radiatum* likewise is purely *I. speciosum*, and so also, doubtless, is his var. *genuinum* itself.

CHICAGO NORMAL COLLEGE
CHICAGO, ILL.

EXPLANATION OF PLATES XXIII, XXIV

PLATE XXIII

Isostigma acaule: a, b, leaves, ×0.54 (type in Herb. Kew); *I. simplicifolium: c, d, e, f,* leaves, ×0.54; *v,* ray floret, ×4.82; *w,* disk floret, ×4.82 (*c, e, f, v, w* from *Sello* 873, type, in Herb. Berl.; *d* from *Claussen*, Minas Geraes, in Herb. Kew); *I. scorzoneraefolium: g, h,* leaves, ×0.54 (from *Riedel*, type of *I. glycinaefolium* in Herb. Kew); *I. Riedelii: i, j, k, l, m,* leaves, ×0.54; *u,* head with upper portion of peduncle, ×0.54; *x,* ray floret, ×3.75; *y,* disk floret, ×3.75 (*i, j* from *Hassler* 9238 in Herb. Berl.; *k, l, m, idem* 10567 in Herb. Berl.; *u, x, y, idem* 8047 in Herb. Berl.); *I. brasiliense: n, o, p, q, r, s, t,* leaves, ×0.54 (*n, o* from *Riedel*, type of *I. stellatum* in Herb. Kew; *p, q, r* from *Glaziou* 21557 in Herb. Berl.; *s* from *Gardner* 4253, type in Herb. Kew; *t, anon.* 98 in Herb. Deless.).

PLATE XXIV

Isostigma dissitifolium: a, j, leaves, ×0.58; *f,* achene, ×0.58 (*a* from *Balansa* 907, type in Herb. Kew; *j, f* from *Fiebrig* 450 in Herb. Berl.); *I. crithmifiolum: b, c,* leaves, ×0.58 (from *Sello* in Herb. Berl.); *I. speciosum: d, e,* leaves, ×0.58 (from *Sello* in Herb. Berl.); *I. megapotamicum: g,* leaf, ×0.58; *k, l,* achenes, ×3.5; *m, n,* flowering and fruiting heads, ×0.58; *v,* ligneous base of stem, ×0.58 (*g* from *Sello* in Herb. Deless.; *k* from *Hassler* 10366 in Herb. Hassl. in Herb. Deless.; *l, m* from *Sello*, *n, v* from *Sello* 1723, in Herb. Berl.); *I. Hoffmannii: h, s,* leaves, ×0.58; *p, q,* disk florets, ×4.65 (*h* from *Lorentz* in Herb. Berl.; *p, q, s* from *Kuntze*, type sheets in Herb. Berl.); *I. Herzogii: i,* entire type fragment, ×0.58; *o,* ray floret, ×4.65; *r,* disk floret, ×4.65; *t,* interior involucral bract, ×4.65; *u,* exterior involucral bract, ×4.65 (all from *Herzog* 617 in Herb. Hassl. in Herb. Deless.).

[13] *K. Fiebrig* 810, dry elevation at edge of valley, etc., Cerros de Tobati, Paraguay, January 31, 1903 (Herb. Berl., 2 sheets; Herb. Field Mus.; Herb. Gray; Herb. Hassl. in Herb. Deless., type of *I. peucedanifolium* var. *genuinum* f. *discoideum* Hassl.); *Hassler* 5586, on high plateau and slopes, Sierra de Maracayú, Paraguay, December, 1898 (Herb. Hassl. in Herb. Deless., type of *I. peucedanifolium* var. *genuinum* f. *radiatum* Hassl.) *idem* 9238, in dry fields near Caaguazu, Paraguay, March, 1905 (Herb. Hassl. in Herb. Deless.).

PENETRATION PHENOMENA AND FACULTATIVE PARASITISM IN ALTERNARIA, DIPLODIA, AND OTHER FUNGI[1]

PAUL A. YOUNG

(WITH PLATES XXV–XXVII)

Introduction

Between the extremes represented by obligate parasites and obligate saprophytes are numerous facultative parasites which attack other organisms with varying degrees of virulence. Most species of *Alternaria* are facultative parasites. This problem was undertaken to determine the penetration phenomena and experimental host ranges of many dematiaceous and some other fungi. Eighty-eight isolations of fungi and two of bacteria were used in the random inoculations of seventy-eight species and varieties of flowering plants.

Some dematiaceous fungi, *Diplodia*, *Cephalosporium*, and *Colletotrichum*, induced wheat coleoptiles to produce thickenings in the cell walls. The term "callosity" is used to designate these local additions to host cell walls caused by stimulation by a penetrating fungus. The illustrations of the kinds of callosities define them best (figs. 1, 2, 5, 6, 8, 9, 11, 16, 21, 22, 24, 26, 27, 30, 32, 34, 36*A*, 37, 38).

With few exceptions, each callosity incloses a prominent structure resembling a brightly shining dot or slender, angularly irregular line which has been termed the "penetration hypha" (figs. 1, 2, 4, 5, 6, 7, 9, 10, 11, 13, 16, 20, 21, 24, 26, 30, 34, 37, 38). Many direct connections between appressoria and callosities were seen.

The appressoria of *Alternaria* consist of enlarged tips of hyphae

[1] Abstract of a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree at the University of Illinois. The cross inoculations will be considered in greater detail in another article.

The author wishes to thank Dr. F. L. STEVENS for proposing the problem and for numerous helpful suggestions. Thanks are also due to Drs. WILLIAM TRELEASE, W. B. McDOUGALL, C. F. HOTTES, Mr. L. R. TEHON, and Mr. A. L. HAFENRICHTER of the Botany Department of the University of Illinois, and all others who have aided in the work on this problem.

or their branches; sometimes of enlarged cells in the hyphae (figs. 7, 13, 16, 20, 22, 24, 27). According to BÜSGEN (3), formation of appressoria occurs in many fungi, and is mainly due to stimuli resulting from contact of the germ tubes with solid bodies.

"Vertical cell walls" refer to the cell walls which appeared to be vertical when the surfaces of strips of epidermis were microscopically examined. Unless otherwise stated, wheat means Red Wave wheat. Most of the inoculations in tubes were made on seedling stems; most of the greenhouse inoculations were made on leaves.

Methods

Fungi were grown on corn-meal agar. Seeds were surface sterilized with a 3 per cent solution of Chloramine-T or a 0.5–1 per cent solution of Uspulun. Seeds were transferred directly from the disinfectants to the wet filter paper in the bottoms of large, autoclaved dishes of the Petri dish type. The dishes were then kept in a dark incubator at 25° C., until the plumules of the seedlings were 1–4 cm. long. Usually within 3–5 days, plumules of wheat, rye, barley, oats, sorghum, and broom corn reached a length of 1–3 cm. without the coleoptiles being ruptured. Uninjured seedlings were inoculated and grown according to the method used by STEVENS (17). Only aseptic seedlings were used. Spores or agar bearing mycelium of fungi were placed on the epidermis of the stems of aseptic seedlings, which were then rolled in wet, autoclaved rags and placed in large tubes. Seedlings in tubes were grown in the light at about 25° C. for 2–14 days after inoculation. Check seedlings remained aseptic for a week or often longer. Wheat seedlings began to show symptoms of drought and starvation injury after they had been in the tubes about 10 days.

Within 2–14 (usually 3–8) days after inoculation, seedlings were examined by the following method. The point of a needle was inserted under the outer 1–3 layers of epidermal cells of an inoculated region. In the case of wheat, a free end was grasped with forceps and a thin strip pulled off. In the case of cabbage, it was necessary to slide the needle under the strip to be removed before the ends were broken loose. Strips were usually stained with acid fuchsin or Congo red, and mounted in glycerine on slides, which were then sealed with gold size. Microtome sections were stained with Pianeze III B.

Spores or agar bearing mycelium of fungi were placed in drops of water on uninjured leaves of greenhouse plants, which were then covered for usually 3 days with bell jars lined with wet filter paper. The bell jars were covered with heavy burlap sacks to prevent sun scalding. Spores usually floated on the drops of water, and were often arranged in rings marking the margins of the layers of water when these drops were covered with cover glasses.

Potted plants were inoculated and placed in large glass cases into which water was sprayed for three days. Such inoculations usually did not result in infections.

Host reactions to cross inoculations

Nearly all the details from 77 tables of cross inoculations are omitted here; some of them will be given in another article. Penetration phenomena are grouped according to hosts.

Triticum aestivum.—All species of *Alternaria*, *Acrothecium*, and *Helminthosporium* placed on wheat coleoptiles caused them to form callosities; most of them caused brown cells. They usually altered regions in cell walls, which appeared as disks or rings when the strips were stained. *Diplodia zeae* (Schw.) Lev., *Cephalosporium acremonium* Corda, and *Colletotrichum nigrum* E. & H. also caused wheat coleoptiles to form callosities.

Infection of wheat coleoptiles by *Alternaria* and *Helminthosporium* results in similar penetration phenomena, so reference is made to the illustrated account by STEVENS (17) of *Helminthosporium* penetration. MANGIN (10) seems to deserve credit for first illustrating and describing the bodies called callosities; he reported them in wheat infected with *Septoria*. FRON (6) illustrated callosities caused by *Leptosphaeria* in wheat. Comparable bodies were described by RAVN (14), DASTUR (4), BREFELD (2), and WOLFF (19). NEGER (12), HIGGINS (8), and SMITH (16) say that hosts form sheaths around the penetrating hyphae of the Erysiphaceae and Coccomyces.

Spores of *Alternaria* and *Helminthosporium* were germinated in water under cover glasses on slides, and stained with dilute gentian violet. Some of the germ tubes in each case showed sheaths. Contact of the germ tubes of these fungi with glass did not cause them to form appressoria.

Examination of wheat coleoptiles within 48 hours after inoculation with *Alternaria* often shows germinated spores bearing appressoria and pegs which have caused the host to produce small callosities; some brown cells may be visible at this time. Stains show that the epidermis near the points of incipient penetration has been greatly altered. The magnitude of the symptoms and accompanying fungous development increases, so that strips of epidermis examined a week after inoculation generally exhibit most of the effects described later; strips removed two weeks after inoculation often show no more than these.

On the lower sides of appressoria appear slender outgrowths (penetration hyphae) which enter the cuticle of the epidermal cells. Wheat cells react to this penetration by the formation of callosities around the penetration hyphae. Each callosity incloses a prominent body which resembles a brightly shining dot or an angularly irregular line which is the penetration hypha. In most cases the callosities increase in size more rapidly than the penetration hyphae, and so continue to inclose them. In many cases, however, the penetration hyphae project from the callosities and cross 1–3 host cells (figs. 2, 9, 13).

Wheat callosities are usually hemispherical in outline and have distinct margins; they are often elongate and sometimes radiate, toothed, or irregular in shape (figs. 1, 2, 5, 6, 8, 34, 38). Callosities often formed dense fields and were frequently packed together; appressoria were usually attached to most callosities, unless torn away. *Diplodia* and *Cephalosporium* induced the formation of circular callosities which were smaller and elongate callosities which were narrower than those caused by *Alternaria;* they were usually more numerous in a unit area. Numerous cases of appressoria attached to callosities by penetration hyphae were seen (figs. 13, 16). Internal hyphae often sent batteries of penetration hyphae into adjacent cell walls and induced many callosities to form; one internal hypha of *Helminthosporium gramineum* sent out a row of 35 penetration hyphae with many more projecting from neighboring internal hyphae. Internal hyphae of *Alternaria* were seen to send out smaller numbers of penetration hyphae. No *Alternaria* penetration hypha was observed to become an internal hypha.

In areas of penetration of wheat and other hosts, the cell walls

were much thickened and exhibited prominent middle lamellae (figs. 1, 2, 3*E*, 4, 5, 9, 10, 12, 13, 16, 24, 28). *Alternaria* species from asparagus and rose produced hyphae in pockets in swollen vertical cell walls of wheat (fig. 12). Penetration hyphae of *Alternaria* usually entered vertical cell walls; ADERHOLD (1) and STEVENS described this phenomenon in penetration by other fungi.

Most of the wheat strips were stained with acid fuchsin or Congo red. Brilliant red rings or disks around the points of incipient penetration showed that the cell walls had been greatly altered at these points.

Yellow, auto-stained disks were seen in the cell walls of one wheat coleoptile infected with *Alternaria;* a gray, auto-stained disk was seen in a coleoptile infected with *Acrothecium*. KLEBAHN (9) described auto-stained disks in tomato stems infected with *Didymella*. Stained disks of altered cell wall material were illustrated by MANGIN (10) and STEVENS (17). EDSON (5) says that the first visible indication of alteration of beet cell walls infected with *Phoma* is a change in their reaction toward the stain.

The red stained rings in wheat coleoptiles caused by *Alternaria*, *Helminthosporium*, *Cephalosporium*, or *Colletotrichum* may be divided into four regions (fig. 4): (1) the callosity itself is usually unstained; (2) the adjacent region is lightly or not at all stained (deeply stained in red disks); (3) a broad, red band surrounds the first two regions and forms the ring; and (4) the unstained, normal host cell walls lie outside the ring. Perhaps some chemical diffused from the penetration hypha in the callosity into the cell wall and caused differential chemical alteration of the wall in different places. It is also possible, although it seems less likely, that physical stimuli from the penetration hypha may have induced physical alteration in the cell wall such as porosity, and thus affected the retention of the stain in some places. Microchemical proof is lacking. These areas, when stained with Pianeze III B, exhibit green centers surrounded by purple borders (fig. 11). A strip of wheat coleoptile infected with *H. gramineum* exhibited blue-purple disks when stained with gentian violet.

Chloro-iodide of zinc (made by dissolving Merck's zinc chloroiodide powder in a volume of water smaller than itself and adding

an excess of iodine crystals to make a red solution) caused brilliant, yellow-brown disks to appear around points of incipient penetration in wheat coleoptiles infected with *Alternaria;* the callosities became dark yellow-brown, but these colors faded within a few days. The inner lamellae of swollen walls became brown when treated in this way. Treatment of infected wheat strips with phloroglucin and hydrochloric acid caused bright red rings to appear around the incipient infection points; the rings soon faded.

Callosities turned brown when treated with nitric acid. Boiling in water did not affect them. Wheat strips were soaked in acetone and others in ether for 5 days without apparent changes in the contained callosities. Wheat callosities were not discolored by iodine dissolved in potassium iodide solution, nor with this treatment followed by concentrated sulphuric acid. These microchemical and staining tests were too incomplete to justify conclusions concerning the chemical nature of callosities, or the nature of the alteration of the cell walls around infection points.

Brown cells occur singly or in small groups in wheat coleoptiles infected with *Alternaria;* browned regions of cells are often large enough to be macroscopically visible as brown streaks. Coleoptiles often become entirely brown, rotten, and bear aerial mycelium; only rarely can thin strips be removed from such coleoptiles. *Helminthosporium* from barley produced new sporophores and spores on wheat coleoptiles in tubes within 4 days after inoculation. Most of the wheat strips were mounted in glycerine. Normal cells were badly plasmolyzed, while brown cells apparently were unaffected by this treatment.

It was tentatively assumed that *Alternaria* and *Helminthosporium* possessed chemicals which altered wheat cell walls and induced callosities to form. To try to determine whether or not such chemicals could enter wheat coleoptiles in the absence of mycelium, *Alternaria* (*Macrosporium avenae* Oud.?) and *Helminthosporium gramineum* were grown in nutrient broth of the following formula: 20 gm. of Difco bacto-beef, 1 gm. of $FeSO_4.NH_2SO_4.6H_2O$, 10 gm. of cane sugar, 7 drops of lactic acid, and 400 cc. of distilled water. A second formula was used later for growing the *Alternaria:* 10 gm. of bacto-beef, 3 drops of lactic acid, and 250 cc. of distilled water.

The mixtures were boiled for a few minutes, filtered, placed in Kolle flasks, and autoclaved. After the fungi had grown on these media for 2–4 weeks, the liquids remaining in the flasks were aseptically filtered through two layers of filter paper. The cloths surrounding aseptic wheat seedlings were saturated with the filtrates and the plants grown in tubes for a week. Liquid pressed from the sectioned mycelial mat of *Alternaria* was also used in this way. In no case did any of the wheat coleoptiles exhibit callosities or red stained rings. These repeated results with no exceptions lead to the conclusions that such penetration phenomena are not due to chemicals from the fungi, or that such chemicals do not penetrate wheat coleoptiles in the absence of mycelium.

It is generally assumed that mechanical injuries of plants cause discolorations. This assumption was proved to be true in the cases of wheat, sorghum, and broom corn. Aseptic seedlings of these plants were repeatedly wounded with the point of a sterile needle and the plants grown in sterile tubes for several days after wounding. Strips from the wounded coleoptiles showed yellow discoloration in wheat, and red-brown discoloration in sorghum and broom corn, in the cells surrounding the wounds (fig. 15). No callosities, autostained disks, or red rings appeared. Since fungi and bacteria were absent, these results prove that mechanical injuries (including breaks) induce these seedling plants to produce intense discolorations in the injured regions. PARKIN (13) described discoloration of *Jacobina*.

Diplodia zeae induced wheat coleoptiles to produce very dense fields of yellow, elongate and circular callosities (fig. 34). Numerous long penetration hyphae occurred. A few elongate callosities were broken out of some of the ruptured wheat cells in an inverted strip. These observations show that elongate callosities are attached by one or both ends; that the centers of such callosities may be free from the cell walls; and that elongate callosities sometimes project down into cell cavities.

Coleoptiles of wheat badly infected with this fungus showed intense browning of entire coleoptiles which bore abundant, white aerial mycelium on the brown regions. Abundant internal mycelium and some radiate callosities were present. Usually 100 per cent in-

fections occurred with this fungus in the five series of inoculations with it.

Cephalosporium acremonium induced wheat coleoptiles to produce great numbers of usually hyaline callosities, which resembled those caused by *Diplodia* in shape and density of fields; some appressoria were seen in contact with callosities. Many inoculations of wheat in the five series failed to result in infections; infected coleoptiles were usually slightly or not at all discolored. Many red stained rings and disks, and some large, complex callosities were seen; Congo red was retained around the penetration hyphae in the callosities in some cases. Many long, freely projecting penetration hyphae were observed. One slide showed two small spots, each of which bore hundreds of penetration hyphae. In one case the spot appeared to be a mycelial mass, perhaps a complex appresorium, which was thickly studded with pegs, and from which a row of many penetration hyphae projected and caused callosities in an adjacent cell wall. Much internal mycelium was seen in one strip; some ladder mycelium passed from cell to cell in a manner resembling that shown in fig. 36. Many brown cells seemed to be nearly full of mycelium. REDDY and HOLBERT (15) discuss this fungus.

Colletotrichum nigrum induced the formation of callosities and altered regions in wheat cell walls. These phenomena often occurred under appressoria (fig. 10). One of the many very large red disks exhibited three appressoria with many radiate callosities in the cells below them. Other wheat strips showed great numbers of red rings, some of which contained minute, circular callosities; each of the callosities and many of the red rings exhibited brightly shining dots representing the penetration hyphae. The strips in another series of inoculations were more heavily infected; some of them bore abundant internal mycelium and superficial acervulae containing many spores. HASSELBRING (7) described the appressoria of anthracnose fungi. Many inoculations of wheat with this fungus failed.

Acrothecium and *Macrosporium cucumerinum* E. & E. induced wheat coleoptiles to form callosities; the penetration phenomena were similar to those caused by *Alternaria*. MITRA (11) says that *Acrothecium* penetrated *Pennisetum* cuticles.

Internal hyphae of *Alternaria* in wheat were usually brown

(sometimes hyaline), and consisted of single filaments, or more often of bands or ribbons, probably formed by the lateral fusion of two or more hyphae. The diameters of the cells of internal hyphae were often greater than those of normal superficial hyphae. Longitudinal sections of seedling wheat stems infected with *Alternaria* (*Macrosporium iridis* C. & E.) showed compact masses of hyphae deep in the host tissues (fig. 3). Callosities occurred in cell walls below the internal mycelium of *Alternaria* in one coleoptile. *Alternaria* sp. from asparagus produced broad bands of brown, internal hyphae (fig. 33*A*).

The superficial hyphae of three species of *Alternaria* became aggregated into compact, brown, fan-shaped masses on wheat and pumpkin seedling stems. STEVENS pictured similar masses of *Helminthosporium* mycelium. The smaller aggregations of superficial hyphae formed tangled masses.

Alternaria sp. from apple surface rot produced very long penetration hyphae in wheat (figs. 2, 13). Internal mycelium of *Alternaria* sp. from rose passed from cell to cell in a seedling wheat stem like the mycelium shown in fig. 36. One germ tube of *Alternaria brassicae* (Berk.) Sacc. f. *microspora* P. Brun. caused eight callosities in a wheat coleoptile like those shown in fig. 5.

No infections resulted when wheat coleoptiles were inoculated with species of *Penicillium*, *Aspergillus*, *Sterigmatocystis*, *Syncephalastrum*, *Phytophthora*, *Saprolegnia*, *Cunninghamella*, *Botrytis*, *Sclerotinia*, *Cephalothecium*, *Fusarium*, *Epicoccum*, *Thielavia*, *Thielaviopsis*, *Pestalozzia*, *Chaetomium*, and two isolations of white-colony bacteria. Species of *Alternaria* from wheat, corn, and cabbage did not cause wheat leaf spots; *Alternaria* (*Macrosporium avenae*) caused a few wheat leaf spots. *Helminthosporium gramineum* caused green infection spots to appear in wheat leaves which had turned yellow under the bell jar (figs. 35*A*, *B*).

Avena sativa, *Secale cereale*, *Hordeum vulgare*, *Zea mays* var. *everta*, and *Z. mays* var. *indentata*.—The reactions of oats, rye, barley, pop corn, and field corn to penetration and infection by *Alternaria* and *Helminthosporium* were similar to wheat reactions. Five isolations of *Alternaria* induced callosities and produced internal hyphae in oat coleoptiles; three species caused the formation

of large, prominent, yellow, auto-stained disks around incipient infection points in oats. Four isolations of *Alternaria* caused oat leaf spots. *Helminthosporium gramineum* induced the formation of three callosities and caused spots in oat leaves. Regions in a seedling stem of field corn infected with this fungus exhibited one red disk, circular, elongate, and complex callosities, some internal hyphae, some appressoria, and superficial fan-mycelium.

Cephalosporium acremonium caused callosities in field corn leaves. This fungus, *Macrosporium cucumerinum*, *Diplodia zeae*, six isolations of *Alternaria*, and two species of *Helminthosporium* caused corn leaf spots. Seven isolations of *Alternaria* caused pop corn coleoptiles to form callosities; five isolations of *Alternaria* caused leaf spots.

Alternaria sp. from wheat caused some callosities in rye. *Helminthosporium gramineum* caused five green spots in rye leaves that had turned yellow under the bell jar. Two species of *Alternaria* caused callosities in barley coleoptiles.

Holcus sorghum.—Infections of sorghum and broom corn seedlings by *Alternaria*, and also purely mechanical injuries, characteristically resulted in the appearance of deep red-brown discoloration of the injured regions (fig. 15). No callosities or auto-stained disks appeared when fungi were not present. The red-brown pigment was produced abundantly and often diffused from diseased or mechanically injured coleoptiles and stained the surrounding cloths.

Points of incipient infection of sorghum coleoptiles by *Alternaria* were often marked by red-brown (sometimes gray) auto-stained disks (figs. 14, 20). Such disks were caused by five species of *Alternaria* and one of *Helminthosporium*. Twelve isolations of *Alternaria* caused brown callosities in sorghum coleoptiles; nine isolations produced internal hyphae. Sections of a sorghum stem infected with *Alternaria ribis* Bub. & Ranoj.? exhibited mycelium in the four outer layers of cells. Six species of *Alternaria* caused sorghum leaf spots; two species caused broom corn leaf spots. Four species caused callosities in broom corn coleoptiles.

Brassica oleracea, *B. rapa*, and *Raphanus sativus*.—Eleven isolations of *Alternaria* caused circular, yellow callosities in cabbage seedling stems; appressoria were usually present on the callosities.

Hyphae of *Alternaria* sp. from *Abutilon* occurred in swollen vertical cell walls (figs. 18, 19). Nine isolations of *Alternaria* produced internal hyphae in cabbage cells. Three species of *Alternaria* and *Helminthosporium gramineum* caused cabbage leaf spots; six species of *Alternaria* did not cause leaf spots. *Alternaria* sp. from pepper leaf spot caused brown, auto-stained disks in seedling cabbage stems.

Four species of *Alternaria* caused callosities in radish seedling stems, five species produced internal hyphae, and five species caused radish leaf spots. *Alternaria brassicae microspora* and an undescribed species from wheat seeds caused green spots in radish leaves which had turned yellow (figs. 40, 43). Four species of *Alternaria* caused callosities in turnip seedling stems; two of them produced internal hyphae.

Cucurbita Pepo and *Cucumis sativus.*—Five isolations of *Alternaria* and three of *Helminthosporium* caused callosities in pumpkin seedling stems (figs. 27, 32); three of these fungi caused callosities in pumpkin stem hairs (fig. 29). Three species of *Alternaria* and two of *Helminthosporium* produced internal hyphae. Three species of *Alternaria* did not infect pumpkin seedlings. *Alternaria* sp. from *Abutilon* produced abundant mycelium in pumpkin cotyledons (fig. 33). *Alternaria* sp. from gooseberry caused some callosities in a watermelon seedling stem.

Three species of *Alternaria*, two of *Helminthosporium*, and *Diplodia zeae* caused callosities in muskmelon seedling stems; all except the last produced internal hyphae. Ladder mycelium of *H. gramineum* is shown in fig. 36. *Alternaria* (probably *Macrosporium iridis*) produced mycelium in swollen vertical cell walls (fig. 24).

Glycine max, Vigna sinensis, and *Pisum sativum.*—*Alternaria* infection of soy bean seedling stems resulted in the appearance of auto-stained, yellow, granular, disk-shaped spots surrounding points of incipient infection; they seemed to be made up of aggregations of yellow droplets surrounding penetration hyphae. Most of the penetration hyphae entered vertical cell walls. Callosities which often had partially indefinite margins inclosed many of the penetration hyphae (figs. 21, 30). Perhaps the hypha shown in fig. 28 represents a compound appressorium. Three species of *Alternaria* caused callosities and produced internal hyphae in soy bean stems; many

of the infected cells turned brown. *Helminthosporium gramineum* caused a yellow callosity in a wax bean seedling stem; leaf spots of this host were caused by this fungus and *Phytophthora cactorum*. Three species of *Alternaria* did not infect uninjured bean leaves.

Alternaria from radish leaf spots caused cowpea leaf spots; another *Alternaria* and two species of *Helminthosporium* failed to do so. Hyphae of *Macrosporium iridis* entered a garden pea leaf through the cuticle and also through a stoma; leaf spots appeared later. *Helminthosporium* sp. and two species of *Alternaria* also caused pea leaf spots.

Abutilon Theophrasti.—*Alternaria* sp. from pepper leaf spot caused this host to form small, circular callosities; *Alternaria* sp. from wheat produced internal hyphae.

Allium Cepa.—Onion bulbs were surface-sterilized with chemicals, almost vertically severed with a knife, and spores or mycelium placed on the membranes between the scales, which were then forced back together, bound with sterilized cloth or rubber bands, and the bulbs then stored in autoclaved dishes for 3–8 days. The onions sent out roots and leaves and remained in fairly good condition for a week.

Macrosporium iridis and *Alternaria* (probably *Macrosporium parasiticum* Thüm.) penetrated onion membranes by sending hyphae of normal diameter through swollen vertical cell walls (fig. 31).

Batteries of *M. iridis* mycelium passed through greatly swollen, brown, vertical cell walls. Another isolation of *M. parasiticum* produced abundant internal mycelium.

Five isolations of *Alternaria* and *Macrosporium* caused aggregations of irregular particles to appear as granular, disk-shaped, hyaline or brown spots surrounding points in vertical cell walls of onion bulb membranes (fig. 23); slender penetration hyphae were not seen. Ten isolations of *Alternaria* and also *Diplodia zeae* did not penetrate onion bulb membranes. Four species of *Alternaria* and *Helminthosporium gramineum* failed to infect onion leaves.

Lycopersicon esculentum, *Capsicum frutescens*, and *Solanum nigrum*.—Callosities induced by eight isolations of *Alternaria* in stems of tomato seedlings were small, circular in outline, yellow, and often consisted of short, swollen regions in vertical cell walls; each callosity inclosed a penetration hypha. Appressoria were usually

present on the callosities. Many of the tomato callosities caused by *Alternaria* sp. from *Abutilon* resembled small, brown, auto-stained disks; this species exhibited one case of stomatal penetration of a tomato seedling (fig. 17). Five species of *Alternaria* produced internal mycelium in tomato stems.

Alternaria from pepper end rot caused prominent pepper leaf spots. Cone-shaped masses of mycelium of *Macrosporium iridis* were seen in sections to penetrate pepper leaves to a depth of 2–4 cells; similar mycelial masses were seen in *Chenopodium album* L. leaves.

Alternaria sp. from tomato end rot infected many hosts, but did not cause leaf spots of pepper or egg plant. *Alternaria* sp. from pepper end rot caused prominent leaf spots in *Datura tatula; Helminthosporium gramineum* also caused leaf spots. *Alternaria* sp. from tomato caused a green leaf spot in a *Solanum nigrum* leaf which had turned yellow under the bell jar; *Alternaria* sp. from pepper end rot caused leaf spots on this host.

Impatiens pallida exhibited leaf spots caused by five isolations of *Alternaria;* aerial mycelium projected from the lower sides of many of the leaf spots.

Besides those here indicated, hundreds of other cross inoculations were made in the laboratory and in the greenhouse.

Discussion

The two hundred new diseases which appeared in the cross inoculations under the conditions described, exhibited symptoms ranging from isolated callosities and scattered brown cells visible only when microscopically examined, to seedling stem rots and large leaf spots. These diseases appeared under conditions very unlike field conditions, so that they are not apt to become serious. Aseptic seedlings in tubes and plants under bell jars were physiologically different from genetically similar plants growing in fields.

A high percentage of the leaf inoculations in the greenhouse did not result in infections. This does not necessarily mean that the hosts were immune in such cases, even though conditions were supposedly very favorable for infection; inoculations were usually not sufficiently numerous and varied to test immunity.

In the cross inoculations, a fungus was said to be the cause of a

leaf or stem spot when diseased lesions appeared below the spores or mycelium used in inoculation and not elsewhere within 1–5 days after inoculation; many isolations and reinoculations were made. In many cases the inoculation work was too incomplete to prove the causal relationships of the fungi below which the spots appeared. Tube inoculations consisted of pure cultures of fungi placed on aseptic seedlings.

The theory has been proposed that the material formed by hosts around penetrating hyphae performs the function of stopping the growth of the penetrating hyphae which they inclose; this seems to be true of most of the callosities seen. The presence of long penetration hyphae projecting from callosities, however, shows that such callosities failed to stop the growth of the penetration hyphae.

Thousands of the slender penetration hyphae of *Alternaria* were examined. Since no case of one of them becoming an internal hypha was observed, perhaps such a phenomenon does not occur in the penetration of wheat coleoptiles by *Alternaria*. It may be that the growth of the penetration hyphae is retarded, or finally stopped, even after they pass beyond the margins of callosities. The presence of many penetration hyphae projecting from internal hyphae of *Alternaria* and *Helminthosporium* is interpreted to mean that the internal hyphae produced the penetration hyphae.

Alternaria and *Helminthosporium* penetrate and infect plants very rapidly in contrast to the slowness with which many fungi of economic importance enter their hosts and cause diseases. In leaves inoculated with *Alternaria* and *Helminthosporium* under bell jars, leaf spots usually appeared within 1–5 days or not at all. Hypophyllous aerial mycelium appeared on many leaf spots within 3 days after inoculation. *Helminthosporium* sp. from barley produced new sporophores and spores on wheat plants in tubes within 4 days, and on its barley leaf spots within 3 days after inoculation. *Helminthosporium gramineum* produced rings of hypophyllous aerial mycelium on its *Impatiens* leaf spots. This fungus and two species of *Alternaria* caused red infection rings below their rings of spores on sorghum leaves (fig. 41).

Some differences in the infections of wheat by *Alternaria* and *Helminthosporium* should be noted. The appearance of great numbers of callosities and red stained rings or disks is characteristic of

infection by *Alternaria*. In contrast to this, *H. gramineum*, for instance, usually causes the appearance of few or no callosities or red stained rings or disks. The appearance of deeper browning of host cells and the presence of more abundant internal mycelium is characteristic of *Helminthosporium* infection of wheat coleoptiles in tube cultures. STEVENS described infection by *Helminthosporium*.

In the inoculation of wheat coleoptiles with *Penicillium* sp., *Botrytis cinerea*, *Cephalothecium roseum*, and *Epicoccum* sp., each of these fungi produced a few simple appressoria without causing the coleoptiles to show any penetration phenomena. Perhaps this demonstration of attempted penetration represents the manner in which parasitism began in fungi which penetrate directly through epidermal cells of host plants. *Alternaria* appressoria often do not produce penetration hyphae which infect cells below them. A majority of the specialized appressoria of *Colletotrichum nigrum* seen on wheat were not associated with infections.

Alternaria penetration of onion bulb membranes was entirely unlike the penetration of wheat coleoptiles. Penetration was by hyphae of normal diameter, which passed through greatly swollen vertical cell walls. The disk-shaped aggregations of irregular granules lacked prominent penetration hyphae; callosities and red stained rings were not seen.

A pumpkin cotyledon reacted to invasion by *Alternaria* sp. from *Abutilon* mycelium by the production of two boundary layers in the lower part of the cotyledon (fig. 33). Subsequent research gave sections showing similar boundary layers in the upper parts of cotyledons in which no mycelium was present. Few of the thirty inoculations of pumpkin cotyledons resulted in infections. Sections of two of the cotyledons showed abundant mycelium in the boundary layers and in cells not cut off by these layers. Such layers occurred under white regions over the midribs of four cotyledons (both of those on two plants); they appeared to cut off part of the palisade and storage tissues. Sections of brown spots which appeared under mycelium did not contain mycelium or show abnormalities.

Internal hyphae of *Alternaria* often assumed the form of ribbons or bands of laterally fused hyphae (figs. 1, 7, 33*A*). These bands were usually brown (sometimes hyaline), and were made up of ir-

regular cells which were often larger than the cells of ordinary superficial mycelium. Compact, fan-shaped masses of brown *Alternaria* and *Helminthosporium* mycelium occurred both in and on wheat coleoptiles. The prominent radiate appearance of such mycelium suggests the radiate mycelium of the Trichopeltaceae and the radiate perithecia of the Microthyriaceae. WOLF (18) used such a character in classifying *Diplocarpon*. STEVENS pictured fan mycelium of *Helminthosporium*. It does not seem that radiate mycelium proves relationship to the Microthyriaceae.

Summary

1. Mechanical injury alone caused cells of wheat, sorghum, and broom corn to become markedly discolored near the points of injury; such discolored regions lacked callosities, auto-stained disks, red rings, and other infection phenomena.

2. The two hundred new diseases which resulted from the cross inoculations occurred under conditions very unlike field conditions.

3. All species of *Alternaria* placed on wheat coleoptiles induced the formation of callosities; all except three produced internal hyphae.

4. *Alternaria* infection of wheat coleoptiles is characterized by the formation of callosities by the host, and by the appearance of stained rings or disks around points of incipient infection.

5. *Alternaria* caused callosities to appear in seedling stems of wheat, oats, rye, barley, pop corn, sorghum, broom corn, cabbage, radish, turnip, tomato, *Abutilon*, soy bean, wax bean, watermelon, pumpkin, and muskmelon; *Helminthosporium gramineum* and *Cephalosporium acremonium* caused them to appear in field corn.

6. *Diplodia zeae*, *Cephalosporium acremonium*, *Colletotrichum nigrum*, and *Acrothecium* sp. induced wheat coleoptiles to produce callosities.

7. Detached and broken elongate callosities induced in wheat by *Diplodia* showed that elongate callosities sometimes project down into cell cavities, and that they are attached by one or both ends.

8. Different combinations of hosts and fungi resulted in the formation of different kinds of callosities.

9. Penetration hyphae usually entered vertical cell walls.

10. In tube cultures, epidermal cells of hosts below *Alternaria* inoculum often showed isolated groups or single brown cells; internal hyphae were not seen in most of such cells.

11. *Alternaria* spp. from asparagus and rose produced internal hyphae in swollen vertical cell walls of wheat; similar internal hyphae of *Alternaria* sp. from *Abutilon* occurred in vertical cell walls of cabbage, and some were produced in vertical cell walls of musk-melon by *Alternaria* sp. from iris.

12. *Alternaria* and *Helminthosporium* usually penetrated the hosts here considered within 1–5 days or not at all.

13. Sorghum and broom corn seedling stems infected with *Alternaria* often exhibited prominent red-brown, auto-stained, disk-shaped spots around incipient infection points. Auto-stained spots, yellow or gray in color, were seen in the epidermal cells of wheat, oats, tomato, cabbage, and soy bean seedling stems infected with *Alternaria*.

14. Infected regions of sorghum and broom corn always had a prominent red-brown color. The pigment was produced abundantly, and often diffused out of infected or mechanically injured seedlings and stained the rags surrounding them.

15. Rings of spores of two species of *Alternaria* and one of *Helminthosporium* on sorghum leaves caused red infection rings.

16. Species of *Penicillium*, *Botrytis*, *Cephalothecium*, and *Epicoccum* produced appressoria on wheat coleoptiles, but caused no infections.

17. Species of *Syncephalastrum*, *Saprolegnia*, *Cunninghamella*, *Botrytis*, *Cephalothecium*, *Fusarium*, *Thielavia*, *Thielaviopsis*, *Pestalozzia*, *Chaetomium*, *Aspergillus*, and *Sterigmatocystis* did not penetrate wheat coleoptiles.

18. Two species of *Alternaria* and one of *Helminthosporium* caused callosities to form in pumpkin hairs.

19. Soy bean seedling stems infected with *Alternaria* exhibited yellow, disk-shaped aggregations of droplets or granules at points of incipient infection.

20. Stomatal penetration by *Alternaria* occurred in a pea leaf and a tomato stem; no other cases were seen.

21. *Alternaria* penetration of onion bulb membranes consisted of hyphae of normal diameter passing through swollen vertical cell walls.

22. Since broth filtrates in which *Alternaria* and *Helminthosporium* had grown and liquid pressed from *Alternaria* mycelium caused no callosities or altered spots to appear in wheat coleoptiles, it is concluded that chemicals from these fungi do not cause such reactions, or that such chemicals do not enter wheat coleoptiles in the absence of mycelium.

AGRICULTURAL EXPERIMENT STATION
BOZEMAN, MONT.

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EXPLANATION OF PLATES XXV–XXVII

Most of the drawings were made with the aid of a projection machine. Magnifications of most of the drawings are shown on the drawings by brackets and measurements in microns. An oil immersion objective was used in making figs. 1, 3, 6, 7, 8, 10, 11, 12, 16, 17, 18, 19, 23, 24, 28, 29, 30, 31, 33, 33*A*. Unless otherwise stated, drawings represent surface views of seedling stems.

PLATE XXV

FIG. 1.—Wheat coleoptile 14 days after inoculation with *Alternaria* from tomato leaf spot: *M*, internal mycelium with penetration hyphae projecting from it and causing callosities in adjacent cell walls; *T*, toothed callosity.

FIG. 2.—Wheat coleoptile 14 days after inoculation with *Alternaria* from apple rot: *P*, penetration hypha 50 μ long; *C*, callosities; swollen host cell walls with prominent middle lamellae shown.

FIG. 3.—Longitudinal section of seedling wheat stem 8 days after inoculation with *Alternaria* from iris: *M*, penetrating cone of mycelium projecting under edge of broken cuticle and causing distinct bending of cell walls below; three cone-shaped masses of mycelium shown in host cells; *H*, host cell wall.

FIG. 4.—Wheat coleoptile 13 days after inoculation with *Alternaria* from gooseberry, showing red stained area bordering penetration hyphae, slightly stained or clear area outside this, and broad, circular band of altered cell wall material deeply stained with Congo red (represented by stippling); cells outside the ring only slightly stained.

FIG. 5.—Wheat coleoptile showing hypha of *Alternaria* from rye causing swellings and some callosities in 7 vertical wheat cell walls which it crosses (7 days after inoculation); appressoria, penetration hyphae, and prominent middle lamellae shown.

FIG. 6.—Two callosities broken out of cells of wheat coleoptile 8 days after inoculation with *Diplodia zeae;* one callosity bears hairlike projection; callosity on left 27 μ long.

FIG. 7.—Appressoria of *Alternaria* from *Datura* 14 days after inoculation on coleoptile of wheat: circles drawn in appressoria represent tops of penetration hyphae; internal mycelium shown in lower cell.

FIG. 8.—Longitudinal section of seedling wheat stem 20 days after inocula-

tion with *Alternaria* from wheat, showing sections of callosities and swollen wheat cell walls; callosities stippled.

FIG. 9.—Wheat coleoptile 9 days after inoculation with *Helminthosporium gramineum*, showing elongate callosities, *E*, containing prominent penetration hyphae, *P*, some of which have free ends.

FIG. 10.—Wheat coleoptile 5 days after inoculation with *Colletotrichum nigrum:* hypha (*H*) bears the appressorium (*A*); peg from center of appressorium penetrated epidermis and was responsible for the altered staining reaction of disk (R), which retained the Congo red stain; middle lamellae prominent in swollen vertical cell walls.

FIG. 11.—Wheat coleoptile stained with Pianeze III B, 8 days after inoculation with *Alternaria* from iris: green region (*G*), contains many callosities (*C*) and two penetration hyphae (*P*); *V*, purple border represented by stippling.

FIG. 12.—Hypha of *Alternaria* from rose in greatly swollen vertical cell wall (*S*) of wheat, 11 days after inoculation: *M*, internal mycelium; swollen cell wall 6 μ in diameter.

FIG. 13.—Wheat coleoptile 14 days after inoculation with *Alternaria* from apple, showing appressorium (*A*), long penetration hypha (*P*), and complex callosity (*C*).

FIG. 14.—Sorghum coleoptile 7 days after inoculation with *Alternaria* from pepper leaf spot, showing characteristic red-brown, auto-stained disk (*D*) surrounding point of incipient penetration.

FIG. 15.—Sorghum coleoptile 7 days after being punctured with point of sterile needle which made the hole (*H*): cells (*B*) were brown; shortest width of hole is 53 μ.

FIG. 16.—Wheat coleoptile 7 days after inoculation with *Alternaria* from corn leaf, showing *Alternaria* spore, germ tube, appressorium, penetration hypha and callosities; penetration hypha is attached to lower side of appressorium; it grew downward, turned, and penetrated vertical cell wall.

FIG. 17.—Epidermis of tomato seedling 6 days after inoculation with *Alternaria* from squash, showing stomatal penetration: *H*, internal hypha which entered through stoma (*S*).

FIG. 18.—Cabbage epidermis 9 days after inoculation with *Alternaria* from *Abutilon*, showing hyphae in greatly swollen vertical cell wall; stippled region was auto-stained yellow.

FIG. 19.—Like fig. 18: end of hypha (*H*) focused in plane slightly above that of vertical cell wall which apparently grew up and inclosed it; *W*, swollen wall.

FIG. 20.—Sorghum coleoptile 7 days after inoculation with *Alternaria* from pepper leaf, showing brown, auto-stained disk under appressorium and peg.

FIG. 21.—Soy bean epidermis 10 days after inoculation with *Alternaria* from bean, showing granular, yellow, auto-stained disk (*Y*); callosity (*C*) contains penetration hypha.

FIG. 22.—Cabbage epidermis 10 days after inoculation with *Alternaria* from tomato rot, showing *Alternaria* spore with germ tube bearing appressorium which causes callosity in vertical cell wall.

FIG. 23.—Surface view of onion bulb membrane 4 days after inoculation with *Alternaria* from onion leaf, showing granular aggregation of irregular particles (*G*) around point of incipient penetration; circle in center of swollen region in cell wall probably represents penetrating hypha; *S*, germinating spore.

PLATE XXVI

FIG. 24.—Muskmelon epidermis 12 days after inoculation with *Alternaria* from iris, showing internal hypha (*I*) in swollen vertical cell wall in which prominent lamellae (*L*) were seen; since the appressoria focus slightly above the internal hypha, the callosities probably grew up and inclosed them.

FIGS. 25, 26.—Muskmelon epidermis 12 days after inoculation with *Alternaria* from iris, showing callosities (*C*), superficial hypha (*H*), and prominent middle lamella (*M*).

FIG. 27.—Pumpkin epidermis 7 days after inoculation with *Alternaria* from iris: *Alternaria* spore (*A*) produced germ tube and appressorium which caused callosity.

FIG. 28.—Soy bean epidermis 10 days after inoculation with *Alternaria* from bean, showing flattened, brown hypha appressed to epidermis and apparently causing yellowing of region around it (*Y*), and swelling of adjacent cell walls.

FIG. 29.—Pumpkin hair 7 days after inoculation with *Alternaria* from rose bud mold: hypha (*H*) attached to lower side of hair by branch and appressorium (*A*); callosity, (C_1) in hair focused below plane of plasmolyzed host protoplasm (*P*); C_2, small callosity; middle host cell is 40 μ wide.

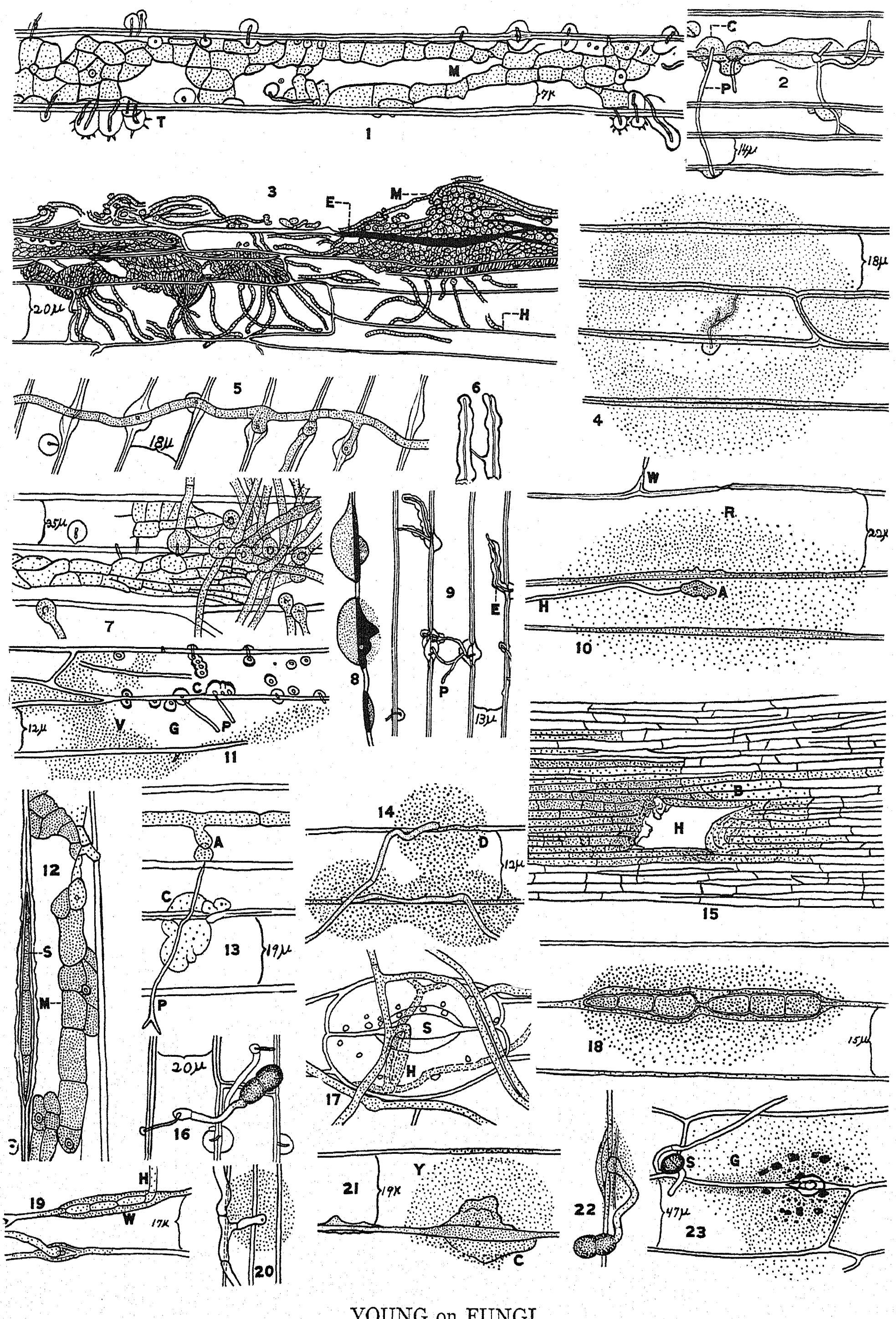
FIG. 30.—Soy bean epidermis 10 days after inoculation with *Alternaria* from bean: *C*, callosity; *Y*, yellow, auto-stained, granular region forming indistinct margin of callosity; *G*, granular type of callosity, including swollen host walls which have prominent middle lamellae; *A*, appressorium with penetration hypha causing callosity; *E*, elongate callosity; stippled regions were yellow.

FIG. 31.—Surface view of onion bulb membrane 4 days after inoculation with *Alternaria* from onion leaf spot, showing penetration by hyphae of normal diameter (*H*), which came from spores (*A*) and passed through swollen region in vertical cell wall (*S*); *M*, superficial mycelium; *N*, host nucleus; *W*, host cell wall.

FIG. 32.—Pumpkin epidermis 7 days after inoculation with *Alternaria* from *Abutilon*, showing callosities (*C*) in region of swollen, yellow cell walls which exhibited prominent middle lamellae and contrasted sharply with the thin hyaline cell walls of normal cells surrounding yellow region; *A*, *Alternaria* spore.

FIG. 33.—Section of pumpkin cotyledon 7 days after inoculation with *Alternaria* from *Abutilon;* dense mass of mycelium (*D*) occupied cavity in lower side of cotyledon and surrounded the small group of tracheae shown above *D;* isolated masses of mycelium (*K*) not yet merged with adjacent mycelial mass (probably because host cell walls had been dissolved away too recently); some sections showed all of outer mycelial mass made up of these blocks of mycelium; *A*, layer of host cells with thickened walls which retained the malachite green

PLATE XXV



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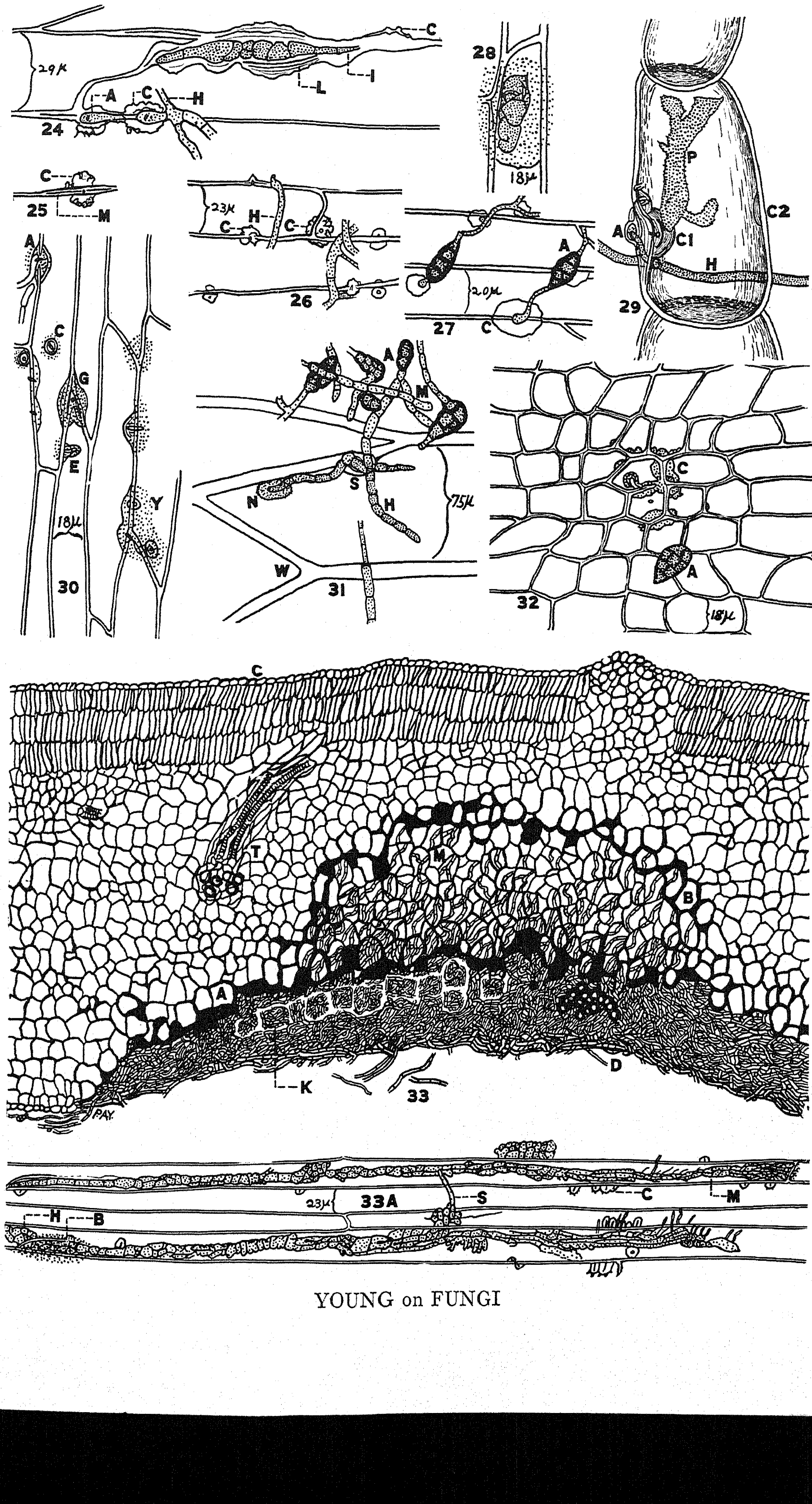

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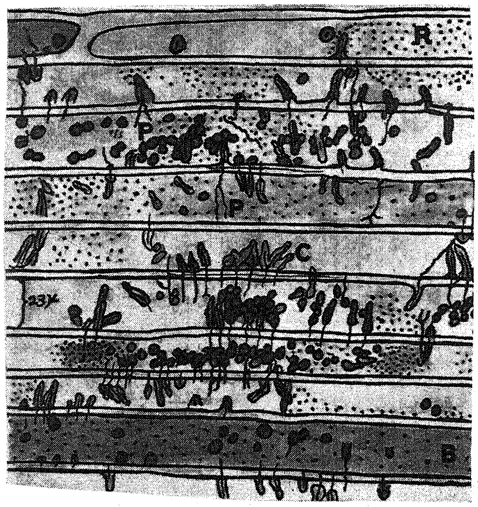

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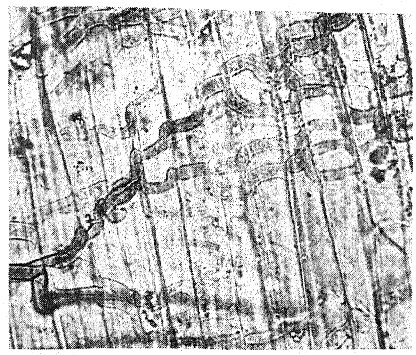

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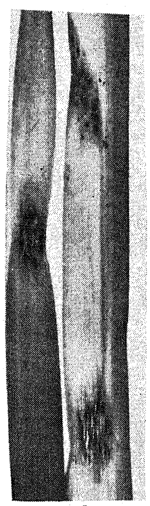
36A



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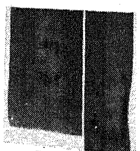
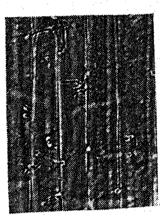


35B



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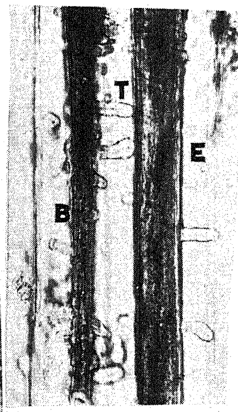

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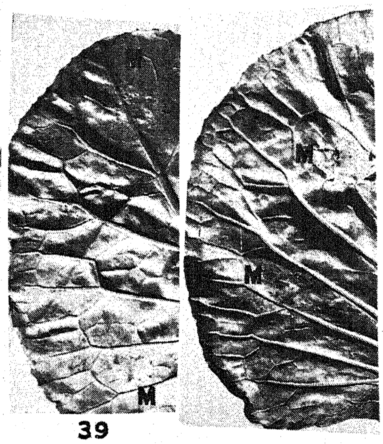

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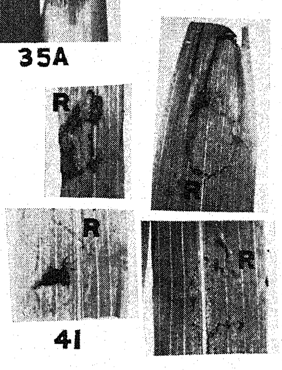

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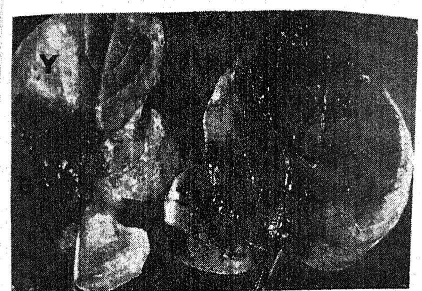

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stain and form outer boundary; *B*, inner boundary; *M*, scattered mycelium in pocket between boundary layers; *T*, tracheae in region uninvaded by mycelium; *C*, cuticle; slightly diagrammatic.

FIG. 33*A*.—Wheat coleoptile 10 days after inoculation with *Alternaria* from asparagus, showing long ribbons or bands of brown, internal hyphae which sent out penetration hyphae; *M*, brown, internal mycelium; *C*, callosity; *S*, superficial hyphae; *B*, brown region of swollen cell wall which partly surrounds internal hypha; *H*, hypha which is probably internal and focuses above hypha inclosed by wall (*B*); slightly diagrammatic.

PLATE XXVII

FIG. 34.—Wheat coleoptile 7 days after inoculation with *Diplodia zeae:* *P*, penetration hyphae; *C*, yellow, elongate, circular, and complex callosities; *R*, plasmolyzed host protoplasm; *B*, brown cell containing callosities.

FIG. 35*A*.—Photograph of wheat leaves 3 days after inoculation with *Helminthosporium gramineum*, showing green leaf spots in leaves which had turned yellow under bell jar.

FIG. 35*B*.—Reverse of phenomenon shown in fig. 35*A;* common yellow spots in green wheat leaves under same bell jar.

FIG. 36.—Photomicrograph of surface of epidermis of muskmelon seedling stem 8 days after inoculation with *Helminthosporium gramineum*, showing ladder mycelium passing through many host cells, chiefly perpendicular to longer dimensions of cells; some hyphae swollen at points of contact with cell walls and constricted in passing through them.

FIG. 36*A*.—Wheat coleoptile 11 days after inoculation with *Alternaria* from *Datura*, showing radiate and irregular callosities (*H*).

FIG. 37.—Photomicrograph of surface of wheat coleoptile 6 days after inoculation with *Alternaria* from gooseberry, showing long penetration hyphae and callosities.

FIG. 38.—Photomicrograph of surface of wheat coleoptile 7 days after inoculation with *Alternaria* from gooseberry, showing plain, elongate callosities (*E*); toothed elongate callosities (*T*); circular callosities; and brown cell (*B*) containing callosities; other brown cell is 25 μ wide.

FIG. 39.—Cabbage leaves 3 days after inoculation with *Alternaria* from tomato end rot, showing hypophyllous aerial mycelium (*M*) projecting from leaf spots.

FIG. 40.—Green leaf spots caused by *Alternaria* from wheat seeds in radish leaves which had turned yellow under bell jar (4 days after inoculation); $\times 0.5$.

FIG. 41.—Sorghum leaves 3 days after inoculation with *Alternaria* from oats, showing red-brown, auto-stained infection rings (*R*) below rings of spores; $\times 1$.

FIG. 42.—Water-soaked region in radish leaf 3 days after inoculation with *Alternaria* from tomato end rot; ring of spores shown; $\times 0.5$.

FIG. 43.—Green leaf spots caused by *Alternaria brassicae microspora* in radish leaves which turned yellow (*Y*) under bell jar; $\times 0.5$.

DEVELOPMENT OF EMBRYO SAC IN GRINDELIA SQUARROSA

THOMAS D. HOWE

(WITH PLATES XXVIII, XXIX AND ONE FIGURE)

Introduction

GUIGNARD (12) was the first to study the embryo sac of a member of the Astereae. In what he referred to as *Conyza ambigua*, he found that the embryo sac develops in the manner typical of angiosperms, from the lower of a row of four macrospores. After the 8-nucleate stage is reached, three antipodal cells are formed. These persist and increase in number as the embryo sac matures, until there is an antipodal group of about ten cells, each with a single nucleus. SMALL (26) evidently questions the identity of GUIGNARD'S plant, since he refers to it as "*Conyza* sp." HALÁCSY (13) cites *C. ambigua* DC. as synonymous with *Erigeron linifolius* Willd. While GUIGNARD does not give the author of his *C. ambigua*, it is probably DE CANDOLLE'S species, since the only other *C. ambigua* cited in the Index Kewensis is a native of South America.

NORRIS (19) found that in *Grindelia squarrosa* (Pursh) Dunal, a typical embryo sac develops from the chalazal macrospore. In the mature embryo sac there are usually two antipodal cells; in rare cases there are three. The lower cell develops a "vermiform extension," which grows through the "endodermis," and which he thought aids in the breaking down of the cells of the integument. It was not figured as extending beyond the first layer of these cells.

MARTIN (18) gave the first account of the embryo sac of *Aster* and *Solidago*. He does not specify from which genus his figures were drawn. The embryo sac develops to the 8-nucleate stage in the typical manner. There are usually three antipodal nuclei, although occasionally four were observed. In the former case, these nuclei are arranged in a triangle with no walls between them. Later stages were not observed.

CHAMBERLAIN (6) studied *Aster novae-angliae*, observing especially the antipodal cells. Contrary to the observations of MARTIN,

he found that the three antipodal nuclei are arranged in a linear row and are separated by cell walls. The antipodal cells increase in number, forming a row of cells, sometimes as many as twenty. This antipodal haustorium extends into the basal part of the ovule. What was thought to be an "antipodal oosphere" was observed in one instance in the basal antipodal cell. This account was confirmed by Miss GOLDFLUSS (11) and Miss OPPERMAN (20). The latter also reported the presence of an antipodal haustorium in *A. undulatus* as well as in *A. multiflorus*. An "antipodal oosphere" with a male nucleus about to unite with it was found in one case in *A. undulatus*. LAND (17), in a study designed primarily to determine the question of the occurrence of double fertilization, reported a varying number of antipodal cells in *Erigeron philadelphicus* and in *E. strigosus*. It seems to have become generally accepted that in the Astereae the antipodal cells persist and develop a chalazal haustorium (COULTER and CHAMBERLAIN 8).

A new interpretation was given by PALM (21) to this haustorial structure. In *Aster novae-angliae* he concluded that the haustorium is formed from persistent macrospores when one of the spores other than the chalazal one develops into the embryo sac. In *Solidago serotina* the micropylar spore is always the functional one, and the others form a haustorium. As evidence, he figures rows of three or four cells with two or four nuclei in the micropylar cell, and later stages showing five or six nuclei in the micropylar cell and one or two in each of the others. In *A. sibericus* the chalazal macrospore always functions. In a later paper (22) he reported that in *A. novi-belgii* the chalazal spore always functions, and that the antipodal cells do not develop a haustorium. In *Bellis perennis* the chalazal macrospore usually develops into the embryo sac. Rarely one of the others functions and the chalazal spore enlarges, forming a cell which CARANO (3) had interpreted as being of antipodal origin. In a publication dealing with the different types of angiosperm embryo sacs (23), PALM gave more facts substantiating his interpretation of this large cell in *Bellis perennis*. In *Emilia* (*Senecio*) *sagittata* he found that any one of the macrospores may function. When the chalazal spore develops into an embryo sac the antipodal cells persist. When one of the other spores functions, the deeper macrospores persist

and enlarge. The cytoplasm of the antipodal cells is denser and less vacuolate than is that of the macrospores. According to AFZELIUS (1), PALM'S plant is really *Emilia amplexicaulis*. CHAMBERLAIN (7) pointed out that the figures given by PALM to support his position are not entirely convincing, and reasserted his opinion that the haustorium in *Aster* and *Solidago* is of antipodal origin.

CARANO (5) studied *Aster novae-angliae* and three species of *Solidago*. In *Aster* any one of the macrospores may develop into an embryo sac, although it is more often the chalazal one which thus functions. The disintegration of the non-functional macrospores was observed in nearly all cases. A few examples were found in later stages of the apparent persistence of a macrospore other than the functional one, but such a persistent macrospore never enlarges to form the haustorium, which latter is always derived from the antipodal cells, at first three in number. CARANO decided that the "antipodal oosphere" is really an enlarged cell of the integument, since in one instance he observed the diploid chromosome number in such a cell.

In *Solidago serotina* and *S. canadensis*, according to CARANO, the chalazal macrospore always develops into the embryo sac. In these species there are at first two or three antipodal cells, although there may be four in later stages. A plant which the writer calls *S. Ridellii* is similar to the others so far as observed. Doubt is expressed as to its identity. In *Bellis perennis* the large cell in the chalazal part of the embryo sac is really derived from an antipodal cell, and not from a persistent macrospore.

In *Erigeron glabellus*, CARANO found many archesporial cells in the nucellus. Each archesporial cell divides, but the second nuclear division is usually not followed by cell division. The lower of the two binucleate cells derived from each archesporial cell develops into an embryo sac, thus following the *Scilla* type of development. The mature sac usually contains two antipodal cells, but they may be suppressed in consequence of competition with neighboring embryo sacs. *Erigeron Karwinskianus* var. *mucronatus* is usually parthenogenetic, and the eight nuclei of the embryo sac are formed by three equatorial nuclear divisions. The number of nuclear divisions is evidently variable, since in one sac ten spindles were observed.

DAHLGREN (9) observed that in *Erigeron aurantiacus* a typical embryo sac is developed. In *Aster capensis* a chalazal haustorium was found, but its origin was not determined. *Chrysocoma coma-aurea* has three antipodal cells, which may be arranged either in a triangle or in a row.

HOLMGREN (15), following a note by TAHARA (28) reporting the occurrence of parthenogenesis in *Erigeron annuus*, studied several species of *Erigeron*. The embryo sac of *E. bonariensis* develops in the typical manner. *E. unalaschkensis* and *E. Coulteri* follow the *Scilla* type of development. In *E. eriocephalus* and *E. politus*, cell division does not occur during meiosis, and a 16-nucleate embryo sac is formed after two more nuclear divisions. The material which he referred to *Erigeron annuus* was somewhat different from the typical form, and cannot be identified with certainty because of the well known occurrence of polymorphism within this genus. The plant is parthenogenetic, and the nuclei of the embryo sac are formed by three equational nuclear divisions in the archesporial cell. The two antipodal cells remain small and later degenerate.

TAHARA (28) published a more complete account of *Erigeron annuus* in connection with the results of studies on *E. linifolius* and *E. dubius*. *E. annuus* is parthenogenetic, the embryo sac nuclei being formed by three equational divisions. The mature embryo sac usually contains only seven nuclei, in consequence of the failure of the chalazal nucleus at the 4-nucleate stage to divide. The two antipodal cells degenerate as the embryo develops. In *E. linifolius* the embryo sac is formed in the typical manner from the chalazal macrospore. After the 8-nucleate stage, two antipodal cells are formed which persist and divide. The two nuclei of the micropylar antipodal cell often fuse before the cell divides, and, since there are nuclear fusions in later formed cells, high chromosome numbers are found at later stages. In *E. dubius* cell walls are not formed between the macrospore nuclei. Usually each of the daughter nuclei formed by their division divides again, the final result being a 16-nucleate embryo sac with eleven antipodal cells arranged in a compact tissue. If some of the nuclei fail to divide, fewer antipodal cells are formed.

PALM (24) found that in *Vittadinia trilobata* the macrospore nuclei are not separated by cell walls, although there is a vacuole

between each two adjacent nuclei. The two chalazal nuclei are slightly smaller than the others, and gradually disintegrate, while the two micropylar nuclei form the embryo sac by means of two successive nuclear divisions. After the second nuclear division, cell plates are formed on the spindles, dividing the sac into six cells. The two antipodal cells persist and increase in number, until there are ten or twelve arranged in a single row.

In 1922 the writer (16) published a preliminary note concerning the embryo sac of *Grindelia squarrosa*. At that time the evidence indicated that the micropylar macrospore develops the embryo sac, and that at least two of the other macrospores persist. It was thought that the non-functional macrospore nearest the embryo sac grows out into the integument, while the other non-functional macrospore enlarges without forming an outgrowth. Later studies have shown that the two cells which thus increase in size are really antipodal cells, and that it is the chalazal macrospore which forms the embryo sac.

Materials and methods

The material used in this investigation was collected near Lincoln, Nebraska, over the period of years from 1920 to 1923. The heads were cut into slices before being placed in the fixing solution. A number of fixatives were tried, and none was entirely satisfactory. A saturated aqueous solution of mercuric chloride with 5 per cent acetic acid gave the least plasmolysis in the developing embryo sac. It obscured the spindle fibers, and after its use staining with safranin was difficult. Flemming's stronger solution gave fair results for the developing macrospores, but it had the disadvantage of affecting the oil droplets so that they took the safranin stain. It was unsatisfactory for later stages. Zenker's solution also gave fair results. Chloroform, xylol, and cedar oil were used to precede infiltration with paraffin. Great difficulty was encountered in cutting the sections because of the hardness of the material. Cutting was more satisfactory after the use of cedar oil. Sections were cut from 5–10 μ thick. Flemming's triple stain and Haidenhain's iron-alum haematoxylin were used. Orange G was sometimes used as a counterstain with the haematoxylin, in order to make the cell walls visible. In the earlier part of the work, gold orange was used instead of orange G because it acted more rapidly.

Macrospore development

The ovule of *Grindelia squarrosa* is similar to the ovules of most other Sympetalae that have been investigated, in having one thick integument. This integument is not distinct on the funicular side of the ovule from the funiculus itself. In the earliest stages studied the integument had nearly grown around the nucellus, which contains one cell larger than its neighbors and with a larger nucleus (text fig. 1*A*). Observations of later stages show that this large cell is the archesporial cell, which functions directly as the macrospore mother cell. The nucellus of this ovule is shown at a greater magnification in fig. 1. No ovules were observed that contained more than one archesporial cell. The nucleus usually passes into synizesis before the integument is fully developed. At this stage there is a typical aggregation of the chromatin threads at one side of the nucleus (fig. 2). In the ovules of one head the nuclei of the archesporial cells were still in the resting condition, although the integuments were fully developed (text fig. 1*B*). One example of the heterotypic equatorial plate was observed (fig. 3). The chromosomes are small, and their number could not be determined with certainty. Since the diploid number is twelve, as will appear later, there are probably six bivalent chromosomes at this time, although only four are distinctly visible on the equatorial plate figured. A cell wall is formed after the heterotypic division, as is usually the case in Compositae (SMALL **26**). The homoetypic mitosis was not observed. That it occurs, however, is shown by the fact that four substantially equal cells are formed which lie in a row (fig. 4). The macrospore tetrad is thus of the type most frequently observed in angiosperms.

Embryo sac development

The chalazal macrospore always develops into the embryo sac. In fig. 6 it is shown in process of enlargement; the other macrospores are disintegrating, as is shown by the homogeneous cytoplasm and by the fact that their nuclei have become dark-staining masses. The cells of the nucellus adjacent to the developing spore are beginning to degenerate. In the section shown in fig. 5, both the chalazal spore and the second spore from the micropyle are enlarging. Fig. 9 shows a later stage in the development of such an ovule, and it is evident in this case that the chalazal spore will develop into an em-

bryo sac. The protoplasm of two of the other spores has lost its structure; and while the nucleus of the second spore from the micropyle is still visible, its reticulum is not seen and its cytoplasm has become coarsely granular. No instances were found in which the chalazal spore was disintegrating. The non-functional macrospores gradually disappear, and all trace of them is lost while the functional one is still inclosed by the nucellus, the cells of which have become structureless masses (fig. 8). The macrospore then, continuing to grow, breaks through the micropylar end of the nucellus, and its later development takes place in contact with the cells of the integument. The remnants of the nucellar cells remain visible about the chalazal portion of the embryo sac until a much later period (fig. 29).

While these changes are taking place, the cells of the inner layer of the integument elongate radially. This elongation is first apparent in the macrospore tetrad stage (fig. 4). The cytoplasm of these cells becomes dense, and they form the "epithelial" layer characteristic of the Compositae. Longitudinal divisions evidently occur, since at later stages the cells of the layer in question are much narrower (fig. 30). Two nuclei were sometimes found in one of these cells, but no evidence of amitosis was observed. The tissue next outside this epithelial layer gradually disintegrates as the ovule matures (fig. 28).

The nucleus of the functional macrospore does not divide until this spore has grown through the micropylar end of the nucellus. Fig. 9 shows a binucleate embryo sac just after nuclear division, the stage being determined by the presence of a spindle. A vacuole appears at each end of the embryo sac, which latter continues to enlarge. One of the daughter nuclei then moves toward the micropylar end of the embryo sac, the vacuole in that part of the sac thus coming to be between the two nuclei (fig. 10). In the section shown in fig. 10, the vacuole has collapsed during fixation. Each nucleus divides (fig. 11), and the daughter nuclei of this division move apart (fig. 12). A vacuole appears between the two chalazal nuclei after this division. Figs. 13 and 14 show each an early 8-nucleate stage, with one spindle still visible in fig. 13 and two in fig. 14. Fig. 14 shows the usual relative position of the egg nucleus and the upper polar nucleus with a cell membrane between them. The embryo sac when fully mature contains six cells, the upper of the two antipodal cells containing

two nuclei (fig. 15). In the section represented in this figure the wall of the embryo sac (macrospore wall) is visible at one point where it passes over the end of the degenerating nucellar cells. This wall also appears in the section shown in fig. 31. Cell division between the two antipodal cells and between the micropylar antipodal cell and the primary endosperm cell occurs by means of the formation of cell plates in the corresponding spindles. In the section shown in fig. 13, the phragmoplast extends to the plasma membrane of the embryo sac. Fig. 18 is an enlarged representation of the lower polar nucleus and of what is to be the micropylar nucleus of the upper antipodal cell of the embryo sac shown in fig. 14. The phragmoplast extends to the plasma membrane of the cell, and the completed portion of the cell plate has split. The halves of the cell plate have become separated during fixation. The method of cell division resulting in the formation of the egg and the synergids was not determined. From the position of the two micropylar nuclei shown in figs. 13 and 14, it is evident that they are to be the nuclei of the synergids appearing in fig. 15. Cell walls are formed between the two antipodal cells and between the micropylar antipodal cell and the primary endosperm cell. These walls are not apparent in fig. 15; but since they occur at all later stages (for example, fig. 29), it is probable that they had not yet been formed in the embryo sac shown in the former figure. Definite walls in this position have been figured by previous writers for several composites (Dahlgren 10, Täckholm 27).

The polar nuclei fuse at the micropylar end of the primary endosperm cell in contact with the membrane separating this cell from the egg (fig. 19), their union forming a nucleus that is much larger than the egg nucleus (fig. 20). The synergids extend into the micropyle; each contains a vacuole in the end nearer the egg. The egg is somewhat pear-shaped, with its broader end away from the micropyle. In the drawings the micropylar end of the egg is hidden by the synergids. The egg apparatus resembles that figured by Land (17) for *Erigeron philadelphicus*.

Fertilization

Although the remains of the pollen tube were seen in several embryo sacs (for example, fig. 25), only one case of fertilization was observed (fig. 21). One synergid is destroyed by the pollen tube as it

enters. There seems to be no marked difference in size or shape between the egg nucleus and the male nucleus at the time of their union (fig. 22). According to LAND, this is also the case in *Erigeron philadelphicus*. The primary endosperm nucleus of the same embryo sac (fig. 23) shows a protuberance which quite probably represents the second male nucleus, but this fact could not be determined with certainty. The primary endosperm nucleus divides before the zygote nucleus (fig. 24), and in consequence of repeated nuclear divisions free endosperm nuclei are formed. Cell division in the endosperm does not begin until after the beginning of the development of the embryo (fig. 25). In *Aster* and *Solidago* (CARANO 5) the first division of the primary endosperm nucleus is followed by cell wall formation, and there is no stage at which free nuclei appear in the endosperm. In some other Compositae, however, such as *Helianthus annuus* and *Centaurea scabiosa* (DAHLGREN 10), free nuclei are found after the embryo has begun to develop. According to the usual classification, *Grindelia* is not closely related to those members of the Astereae which have been investigated, so that a different method of endosperm development is not surprising.

Antipodal cells

The nucleus of the chalazal antipodal cell almost always divides. In most cases the cell enlarges and grows outward into the integument. In fig. 29 it is shown growing through the epithelial layer, a stage similar to that figured by NORRIS (19). It often grows into the integument for a considerable distance, forming a lateral haustorium (fig. 31). No instances were found in which it had grown directly toward the base of the ovule to form the more frequently described type of chalazal haustorium. The antipodal cell nearer the micropyle almost always persists, and usually also grows into the integument to form a lateral haustorium. This development takes place at about the time the embryo sac is mature. In fig. 30, showing a part of the same embryo sac represented in fig. 20, this second antipodal cell has just grown through the epithelial layer of the integument. The lateral haustorium or haustoria thus developed extend out into the tissue of the integument for a considerable dis-

tance. The one nearer the micropyle often grows through the degenerating cells surrounding the epithelial layer, into the more solid tissue of the ovule (fig. 27), the degenerating cells being shown in the figure by broken lines. In fig. 26 such a haustorium appears within two cell layers of the surface of the ovule. The direction of growth of this haustorium is usually not strictly perpendicular to the long axis of the embryo sac, but is inclined somewhat toward the micropylar end of the ovule. Fig. 31 shows lateral haustoria from both antipodal cells appearing in the same section. These haustoria may grow into that part of the ovule in which the integument is not distinct from the funiculus, as well as into the integument itself. In practically all mature embryo sacs seen, at least one such haustorium was observed. Several nuclear divisions occur in these proliferating antipodal cells, but cell division was never observed. The cytoplasm of a haustorium is densest near its distal end, and it is in this dense cytoplasm that most of the nuclei are found. In fig. 28 five nuclei are shown near the end of a lateral haustorium derived from the micropylar antipodal cell. Two nuclei are much larger than the other three; and since each has two nucleoli, it might be supposed that they had been formed by the fusion of nuclei. The three smaller nuclei are in close contact, as though they might be in process of fusion. Nuclear fusions in antipodal cells were reported by TAHARA (29) for *Erigeron linifolius*. The cells of the integument adjacent to a haustorium show no more evidences of degeneration than do other cells equally distant from the epithelial layer and not in contact with a haustorium. There is thus no evidence that the haustoria serve as active agents in the disintegration of the cells of the integument. The antipodal outgrowths persist until they are absorbed by the developing embryo.

NORRIS (19) mentions that in rare instances three antipodal cells were found in *Grindelia squarrosa*. No such cases were observed in the material that I have studied.

Several-ovuled ovaries

In two heads of flowers nearly every ovary contained either two or three cavities with an ovule in each. In the section shown in

text fig. 1*C* only one of the ovules appears, the other being in another section. In most of the ovules in these heads the development of the

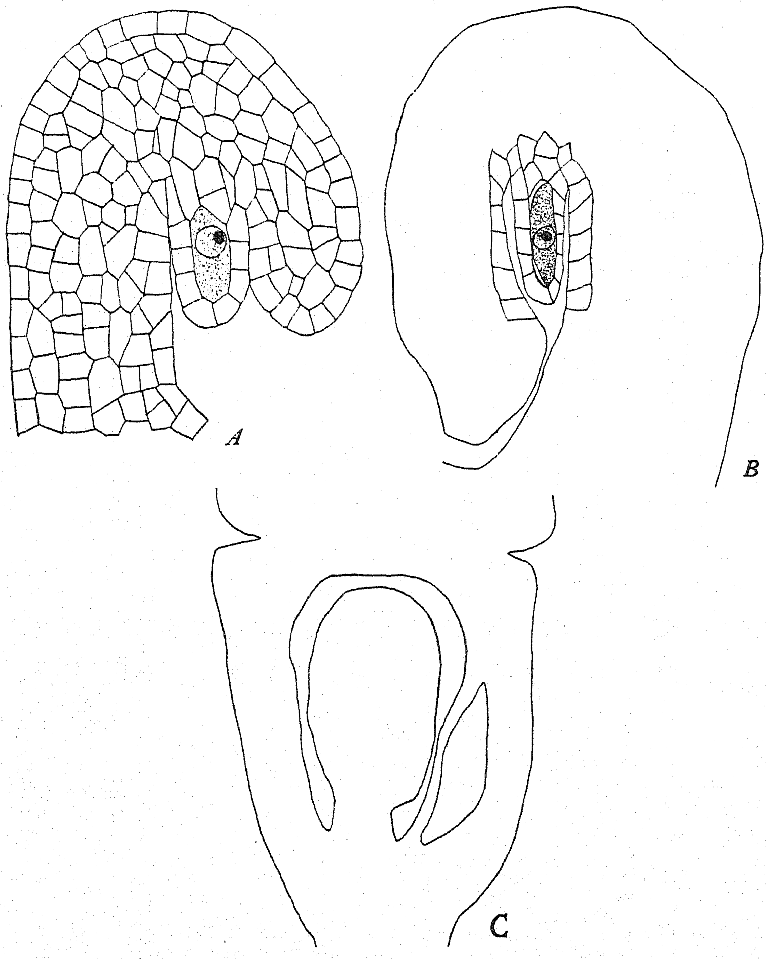

FIG. 1.—*A*, young ovule, showing integument nearly grown around nucellus, which contains archesporial cell, ×330; *B*, unusual ovule, in which nucleus of macrospore mother cell is still in resting condition after integument has grown around nucellus, ×330; *C*, unusual ovary containing two cavities, showing ovule in one; another section shows ovule in the other cavity; ×85.

chalazal macrospore into an embryo sac was in progress. No stages were found showing earlier or later stages of such ovaries. AFZELIUS

(1) found that occasionally the ovary of *Senecio disciflorus* has two cavities, each with an ovule. Miss E. N. ANDERSON[1] found that in various Compositae the ovary has three cavities when the style has three branches, but only two cavities in any case contain ovules. In the present material the number of stylar branches was not determined, since the unusual condition of the ovaries was not noticed until after the sections had been cut.

Chromosome number

Chromosome counts made from mitotic figures in the developing ovules showed twelve to be the diploid number (fig. 16). Division of the nuclei in the embryo sac seems to be nearly simultaneous, since three of the four nuclei were in process of division in the only sac in which mitosis was observed (fig. 17). These figures, while not conclusive in themselves, agree with the expectation that six chromosomes (the haploid number) would be present on the gametophytic spindles. The only mitotic figures seen in the endosperm were of the first division in each of two ovules (fig. 24). These figures indicate that more than twelve chromosomes were present, although the number could not be determined with certainty. These last mentioned divisions thus furnish additional evidence that typical double fertilization occurs.

Discussion

The development of the embryo sac of *Grindelia squarrosa* to the 8-nucleate stage is essentially like that of most other Compositae that have been studied. The brief description of NORRIS (19), so far as it goes, agrees with the account given by the writer. The extensive development of lateral haustoria from the antipodal cells, however, is apparently unlike anything previously reported for any plant, although NORRIS gives one figure of what is probably an early stage in the development of such a structure. Aside from this one doubtful reference, two types of haustoria have been described in the Compositae: those which are derived from antipodal cells and grow directly toward the base of the ovule, and those which are derived from synergids and grow into the tissue surrounding the micropyle

[1] Unpublished thesis, University of Nebraska.

of the ovule. A chalazal haustorium formed by the aggressive development of the antipodal cells has been known since GUIGNARD'S study of *Conyza ambigua*. This structure is especially well developed in *Aster novae-angliae*, where CARANÓ (5) has definitely shown that it is of antipodal origin. A summary of the number and character of the antipodal cells in Compositae which have been investigated is given by SMALL (26).

A micropylar outgrowth in *Calendula arvensis* was first figured by TULASNE (30), who considered it to be an extension of the suspensor of the embryo. HOFMEISTER (14) gave evidence to show that it is really an enlarged synergid. BILLINGS (2) found that in seven species of *Calendula* a similar haustorium occurs, and traced its development from a synergid. This account was confirmed by CARANÓ (4). DAHLGREN (10) found that in *Ursinia anthemoides* the synergids elongate and may form a haustorium which grows into the funiculus at the base of the ovule. Haustoria of this nature are generally supposed to have a nutritive function, breaking down the tissue of the integument and absorbing food substances for the developing embryo. No evidence that they serve as active agents in disintegrating the cells was found in *Grindelia squarrosa*.

SHARP (25) has described a lateral haustorium in *Physostegia*, formed by the endosperm. In this case, however, the haustorium grows toward the base of the ovule and forces the antipodal cells into a lateral position. At least one of the antipodal cells persists, but it does not increase in size.

Grindelia also differs from other members of the Astereae hitherto investigated in that there are at first free nuclei in the endosperm. As already pointed out, this plant is not considered to be closely related to any one of those previously studied.

Summary

1. The ovule of *Grindelia squarrosa* has one thick integument; the nucellus consists of one layer of cells outside the archesporial cell, which latter functions directly as a macrospore mother cell.

2. The macrospore tetrad consists of a row of four substantially equal cells, of which the chalazal one always gives rise to the embryo sac.

3. After the 8-nucleate stage is reached the sac is divided into six cells, the upper (micropylar) antipodal cell having two nuclei. Cell division between the two antipodal cells and between the micropylar antipodal cell and the primary endosperm cell takes place by means of cell plates on the corresponding spindles. Cell walls are later formed between these cells.

4. The two antipodal cells persist but do not divide. One or both of them grow laterally into the integument, thus forming one or two lateral haustoria, which may extend nearly to the surface of the ovule.

5. Typical double fertilization probably occurs. The fusion of the egg nucleus with one male nucleus was observed.

6. The primary endosperm nucleus divides before the zygote nucleus. Several free endosperm nuclei are formed before cell division occurs within the endosperm.

7. Twelve is the diploid chromosome number.

I wish to express my appreciation and thanks to Dr. E. R. Walker of the University of Nebraska, under whose direction this work was begun, and to Dr. C. E. Allen, under whose direction it was completed, for helpful advice and suggestions.

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Decatur, Ill.

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EXPLANATION OF PLATES XXVIII, XXIX

All drawings were made with the aid of a camera lucida. The actual magnification allowing for the reduction is given in each case.

PLATE XXVIII

FIG. 1.—Nucellus, showing archesporial cell (macrospore mother cell); ×820.

FIG. 2.—Synizesis in macrospore mother cell; ×2100.

FIG. 3.—Heterotypic equatorial plate in macrospore mother cell; ×2100.

FIG. 4.—Macrospore tetrad, with surrounding cells of integument showing beginning of elongation; ×820.

FIG. 5.—Macrospores; both chalazal spore and second one from micropyle beginning to enlarge; reconstructed from two sections; ×820.

FIG. 6.—Macrospores, showing chalazal one enlarging and others in process of degeneration; reconstructed from two sections; ×820.

FIG. 7.—Macrospores, chalazal one enlarging and others in process of degeneration; nucleus of second spore from micropyle still visible; ×820.

FIG. 8.—Functional macrospore, after others have entirely disappeared; reconstructed from two sections; ×820.

FIG. 9.—Binucleate embryo sac just after first nuclear division; macrospore has grown through end of nucellus and is in contact with integument; ×820.

FIG. 10.—Binucleate embryo sac after one of daughter nuclei has moved to near micropylar end of sac; remnants of nucellar cells visible at chalazal end of sac; reconstructed from two sections; ×500.

FIG. 11.—Two ends of 4-nucleate embryo sac just after nuclear division; drawn from two sections; ×500.

FIG. 12.—Four-nucleate embryo sac after daughter nuclei have moved apart; ×500.

FIG. 13.—Eight-nucleate embryo sac; remnants of nucellar cells still visible; phragmoplast of spindle of third nuclear division has reached plasma membrane of embryo sac; ×500.

FIG. 14.—Eight-nucleate embryo sac, showing more usual relative position of egg nucleus and upper polar nucleus; phragmoplast of spindle of last division has reached plasma membrane of embryo sac, and cell plate has split; ×500.

FIG. 15.—Embryo sac after all cells have been formed, surrounded by epithelial layer; ×380.

FIG. 16.—Equatorial plate from developing ovule, showing twelve chromosomes; ×2500.

FIG. 17.—Equatorial plate from embryo sac, showing six chromosomes; ×2500.

FIG. 18.—Enlarged representation of lower polar nucleus and one antipodal nucleus shown in fig. 14 showing phragmoplast and split cell plate; ×2500.

FIG. 19.—Micropylar end of embryo sac, showing fusion of polar nuclei; primary endosperm cell separated from upper antipodal cell by cell wall; ×820.

FIG. 20.—Micropylar end of embryo sac after fusion of polar nuclei; ×820.

PLATE XXIX

FIG. 21.—Fertilization, showing fusion of egg and male nuclei, and empty pollen tube: ×1000.

FIG. 22.—Egg and male nuclei fusing; ×2100.

FIG. 23.—Primary endosperm nucleus of same embryo sac of which the egg is shown in fig. 22; protuberance probably represents second male nucleus, ×2100.

FIG. 24.—First division of primary endosperm nucleus; zygote nucleus has two nucleoli; ×2100.

FIG. 25.—First division of zygote nucleus; free endosperm nuclei and remains of pollen tube shown; ×850.

FIG. 26.—End of lateral haustorium from micropylar antipodal cell which has extended to within two layers of cells of surface of ovule; ×500.

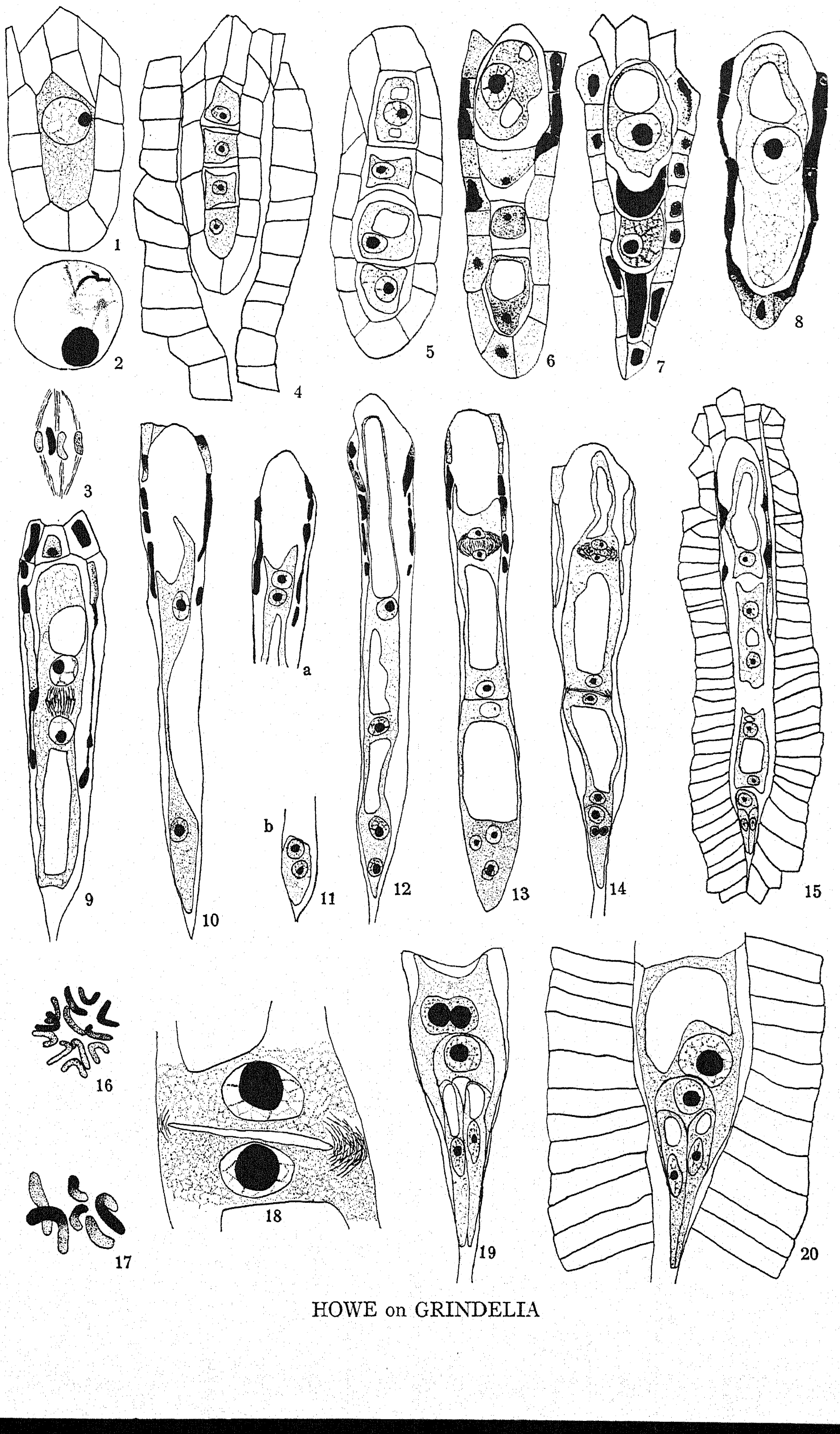
FIG. 27.—End of lateral haustorium from micropylar antipodal cell which has reached the more solid tissue of ovule; three nuclei shown; ×500.

FIG. 28.—Lateral haustorium from micropylar antipodal cell containing five nuclei, two being much larger than other three; ×500.

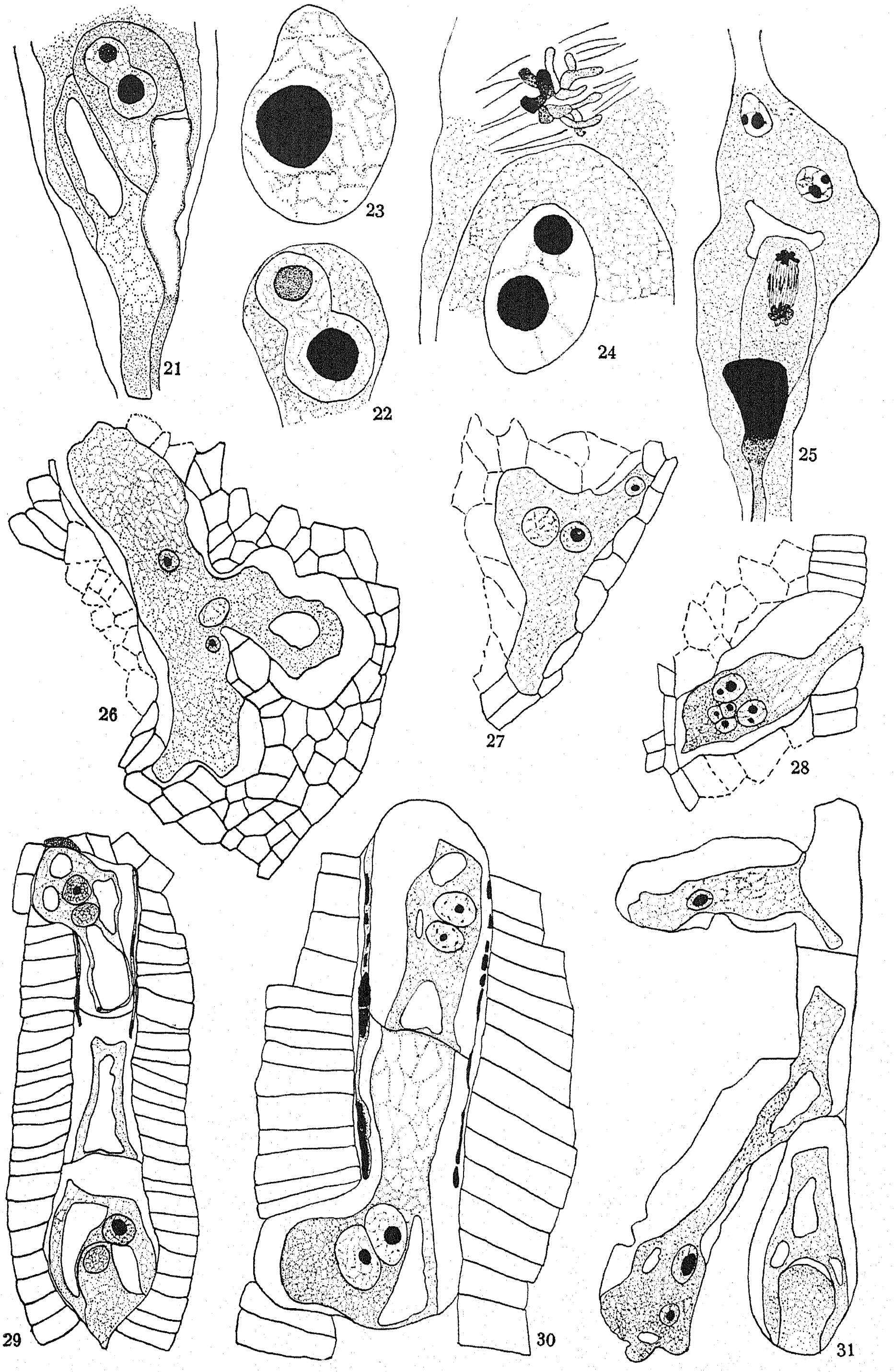
FIG. 29.—Embryo sac in which chalazal antipodal cell has grown through epithelial layer; ×500.

FIG. 30.—Antipodal cells of embryo sac shown in fig. 20; micropylar antipodal cell grown through epithelial layer; remnants of nucellus still visible; ×820.

FIG. 31.—Embryo sac, showing lateral haustoria from both antipodal cells; ×380.



HOWE on GRINDELIA



HOWE on GRINDELIA

EFFECT OF THIOUREA UPON BUD INHIBITION AND APICAL DOMINANCE OF POTATO[1]

F. E. DENNY

(WITH SEVEN FIGURES)

Introduction

At each "eye" of the potato (*Solanum tuberosum*) are the rudiments of several buds (three or more, according to ARTSCHWAGER 3). Usually only one of these buds will develop, the growth of the accessory buds being inhibited. If the sprout which first starts is removed, however, then one of the other buds will begin active growth. Whatever may be the fundamental cause of the repression of these supernumerary buds by the dominant bud, it is shown in this paper that treating the cut tuber with a solution of thiourea (NH_2CSNH_2) causes the growth of two or more (often four or five) buds from a single eye.

The tuber of *Solanum tuberosum* is morphologically a thickened stem, the "eyes" being the much shortened or collapsed lateral branches (APPLEMAN 1). Furthermore, these eyes are located on the tuber in a definitely formed spiral (ARTSCHWAGER 3), comparable to the arrangement on an ordinary leafy twig. In agreement with most stems in young stages, the tubers of most varieties of potato exhibit apical dominance, that is, the ability of the apical bud to prevent the growth of basal buds (APPLEMAN 2). Whatever may be the mechanism by which this dominance of the apical bud is exerted, it is shown in this paper that a treatment with a solution of thiourea prevents the apical bud from completely inhibiting basal buds, and that under certain conditions the direction of dominance may be reversed, so that the apical bud is itself inhibited.

Development of extra buds

From tubers of the Bliss Triumph variety seed pieces were prepared, each weighing about 25 gm. and bearing one eye. They were

[1] Published upon its recent receipt at the expense of the Boyce Thompson Institute for Plant Research.

soaked one hour in a 3 per cent aqueous solution of thiourea, were then rinsed in tap water, planted in soil, and stored in a cool place until sprouting began. The result is shown in fig. 1. It will be noted that out of twenty pieces treated, seventeen or eighteen show the development of more than one sprout per eye, and that in the case of the piece in the upper left hand corner eight sprouts had started.

It is true that not all buds which start will continue to grow, but figs. 2 and 3 show a later development of sprouts from a single eye

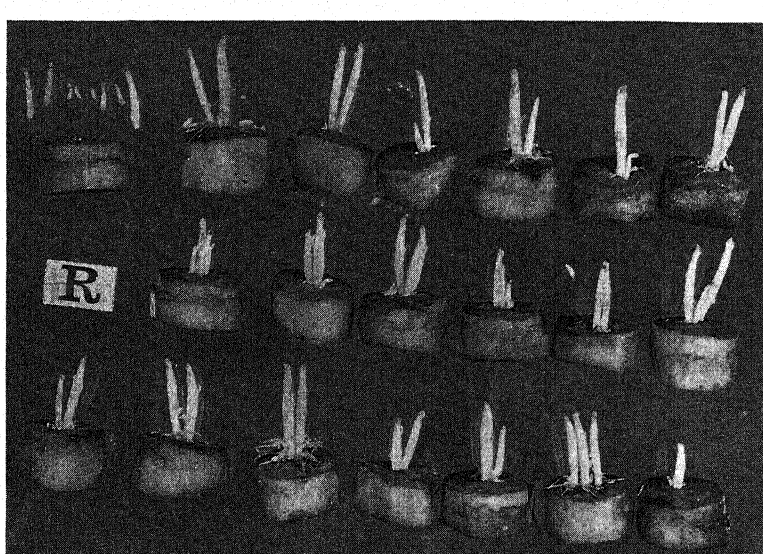


FIG. 1.—Bliss Triumph tubers soaked one hour in 3 per cent solution of thiourea; showing effect of treatment in causing development of more than one sprout per eye.

of another treated tuber. Fig. 3 shows five sprouts that grew from one eye, four of them at approximately the same rate; there is no indication here that one bud is influencing the growth of any other bud, although no doubt the growth of each was being restricted by the lack of sufficient available food in the mother piece. Fig. 3 also shows the development of side branches from a sprout in this early stage of development. With untreated tubers branching does not usually begin until a much later stage of growth. Branching in this case began as soon as the bud emerged from the tuber. It is possible that some of the extra sprouts may be branches from one sprout, but this did not appear to be the case when the cluster of sprouts

was separated. In any event it shows the effect of thiourea upon the course of development of the buds in the eye of the potato.

This multiple sprout effect was obtained with Bliss Triumph, Irish Cobbler, McCormick, and Rural New Yorker varieties. The concentration and time of treatment required to produce it varied with the variety and stage of dormancy, but a suitable range was found to be about 2–4 per cent thiourea, the soaking being continued for one to two hours.

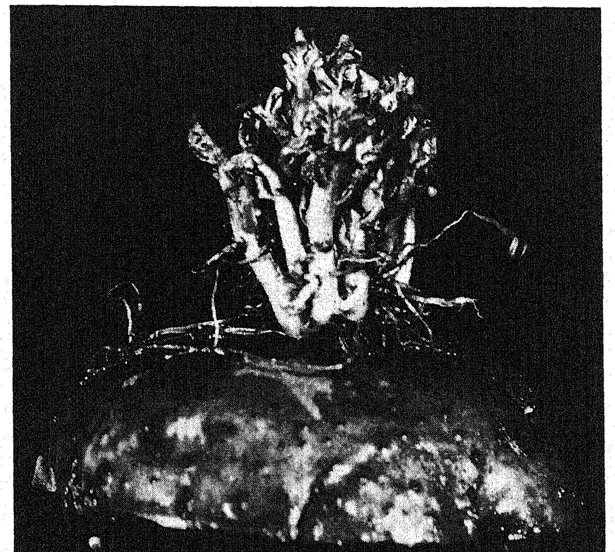


FIG. 2.—Effect of thiourea in inducing formation of several sprouts from one eye of potato (Bliss Triumph); cut tubers soaked one hour in 4 per cent thiourea.

More than 200 chemicals were tried, but thiourea was the only one that consistently forced multiple sprouts without causing rotting of the tubers. As shown in fig. 1, the surfaces of the treated pieces were bright and clear, indicating that no serious injury to the tissue had been caused. In many cases other chemicals caused the development of more than one bud per eye in a small percentage of the pieces treated; but this occurred when the concentration of chemical was high enough to cause considerable injury. In such cases many of the treated pieces rotted either wholly or partly.

The list of chemicals that did not give this multiple sprout response included representatives of various classes of compounds,

both inorganic and organic. Space is not available in this article to publish a complete list of chemicals used, but a few of them may be given, together with a description of the approximate range of concentration and time of treatment. The following were tried by the

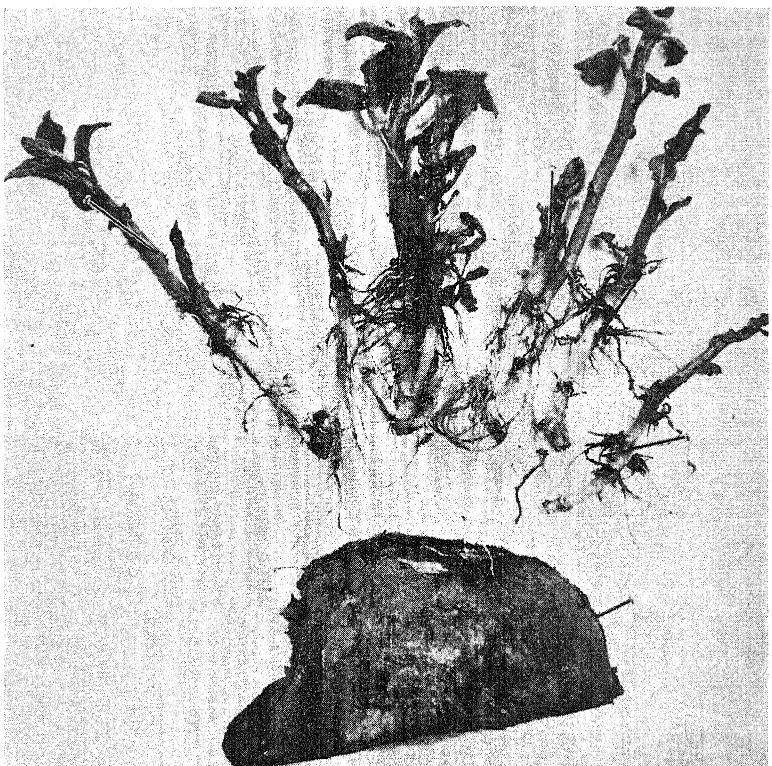


FIG. 3.—All sprouts shown grew from one eye of potato (Irish Cobbler); four out of five growing at approximately same rate; inhibition of growth of subsidiary buds by dominant bud prevented; cut tubers soaked one hour in 4 per cent thiourea.

soak method, the time of treatment being one hour:[2] acetamide 0.1–100 gm.; ethyl alcohol 0.3–9 cc. of 95 per cent; boric acid 0.25–10 gm.; bleaching powder (commercial) 2–80 gm.; chloral hydrate 0.25–10 gm.; ethylene glycol 1–25 cc.; ethylene oxide 0.1–10 cc.; furfural 0.5–20 cc.; pyridine 0.01–10 cc.; phenol 0.01–10 gm.; methyl-urea nitrate 1–10 gm.; mercuric chloride 0.1–1 gm.; manganous chloride

[2] The numbers placed after each chemical show the amounts per liter of water.

0.126–12.6 gm.; potassium permanganate 0.0156–15.6 gm.; sodium nitrate 0.85–59.5 gm.; potassium ferrocyanide 0.6–5 gm.; potassium ferricyanide 1–10 gm.; strontium nitrate 3–30 gm.; sodium potassium tartrate 3–30 gm.; ammonium sulphate 33–66 gm.

The following chemicals failed to induce the development of extra buds per eye by the vapor method, using cut tubers:[3] bromoform 0.02 for 3 hours to 0.3 cc. for 24 hours; chlorobenzene 0.06 cc. for 3–24 hours; ether 0.01–2 cc. for 3 hours; ethylene dibromide 0.001 cc. for 3 hours to 0.01 cc. for 24 hours; ethyl nitrite 0.01–0.3 cc. for 3 hours; gasoline 1 cc. for 1–24 hours; methylene chloride 0.5 cc. for 1–24 hours; picoline 0.01–0.3 cc. for 3 hours; acetylene tetrachloride 0.06–0.3 cc. for 16 hours; trimethylene chlorhydrin 0.1–0.5 cc. for 16 hours; toluol 0.06–0.3 cc. for 3 hours.

Injecting the tissue just below the bud with 1 per cent cane sugar and 3 per cent hydrogen peroxide did not force the sprouting of extra buds per eye; soaking in a complete mineral nutrient solution was also without effect.

A special test was made for the purpose of comparing the effect of thiourea and of other "urea" and "thio" compounds. Bliss Triumph dormant tubers from the 1925–26 crop from Bermuda were used. The potatoes were purchased in the Yonkers markets as soon as the Bermuda crop began to arrive. Because of the small size of some of the tubers the cut pieces did not all have only one eye each; some had two eyes. Table I shows the effect of the treatments upon the number of sprouts per seed piece that had appeared above ground after 35 days.

From table I it is seen that only thiourea showed any marked tendency to force the sprouting of more than one bud per seed piece. Urea was ineffective at the concentrations used, although it is possible that stronger percentages for longer periods of treatment would give better results. This should be tested. Ortho-tolyl-thiourea caused prompt sprouting of dormant buds, but double sprouts started from only one seed piece. Di-ortho-tolyl-thiourea was ineffective either in causing early sprouting or in forcing the growth of extra buds.

In these experiments the thiocyanates were next to thiourea in

[3] The numbers following each chemical show the amount of the chemical used per liter of air space in the container in which the treatment was carried out.

the order of effectiveness in respect to multiple sprout formation. In many experiments, not shown in table I, it was noted that double or triple sprouts from single eyes were not uncommon in treated lots with sodium, potassium, or ammonium thiocyanate. The thiocyanates also caused the development of sprouts that were noticeably plumper and fatter than normal sprouts (fig. 4). Occasionally these sprouts would also become twisted or gnarly. These results, although

TABLE I

EFFECT OF THIOUREA AND OF OTHER "THIO" AND "UREA" COMPOUNDS UPON NUMBER OF SPROUTS PRODUCED PER SEED PIECE OF POTATO

Chemical	Concentration per cent	Duration of treatment (hrs.)	Number of sprouts per seed piece	Chemical	Concentration per cent	Duration of treatment (hrs.)	Number of sprouts per seed piece
Thiourea.......	4	1	2.5	Allyl thiourea...	1	1	1.0
Thiourea.......	2	1	1.8	Phenyl thiourea.	*	1	0.5
Thiourea.......	4	1	3.7	Sodium thiocyanate.........	3	1	1.1
Thiourea.......	4	2	4.0	Ammonium thiocyanate......	3	1	1.2
Thiourea.......	2	1	3.9	Sodium thiosulphate........	8	1	0.1
Thiourea.......	2	2	3.9	Ammonium thiosulphate.....	8	1	Rotted
Urea...........	10	1	0.6	Potassium cyanide.........	0.1	1	0.5
Urea...........	8	1	0.1	Dicyandiamide..	3	1	0.0
Urea...........	2	1	0.3	Ethylene cyanide	0.2	1	0.3
Urea nitrate....	3	1	rotted	Check-H_2O.....	....	1	0.3
Urea nitrate....	1	1	0.2	Check-H_2O.....	....	2	0.9
Urea nitrate....	0.3	1	0.3				
O-tolylthiourea..	*	1	1.1				
O-tolylthiourea..	*	2	1.6				
Di-o-tolyl thiourea..........	*	1	0.2				
Di-o-tolyl thiourea..........	*	2	0.9				

* Water-saturated solution.

frequently obtained, could not be produced at will, as was the case with the thiourea treatments, and therefore less emphasis is placed upon them.

Apical dominance

In order to determine the effect upon apical dominance, the tubers were cut lengthwise, into halves or quarters, depending on the size of the tubers. The cut pieces were then soaked in different concentrations of thiourea for different lengths of time, rinsed, and planted in soil and stored in a cool place. After sprouting began they were placed in a greenhouse.

The results of an experiment carried out in this way are shown in fig. 5. The checks in the bottom row show the normal behavior of Bliss Triumph tubers, three out of five pieces only showing one sprout per piece, and this sprout starting from the apical end. In this photograph the pieces are arranged so that the apical end is directed toward the right.

In the two treated lots (lots *G* and *K*, fig. 5), however, it will be noted that the apical end did not dominate the basal end. In most cases sprouts started from two or even more buds on each piece; in some cases buds started nearly as well from the basal end as from the apical. Several examples of multiple sprouts from single eyes are shown in the treated lots.

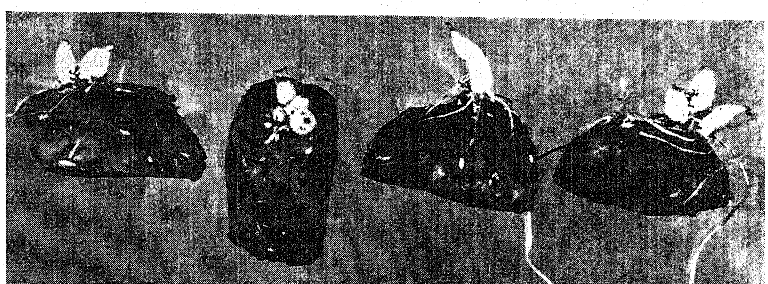


FIG. 4.—Effect of potassium thiocyanate in causing multiple sprouts at eye of potato (Bliss Triumph); note fatness of buds; this result frequently but not always obtained; 3 per cent potassium thiocyanate for one hour.

The behavior of Irish Cobbler and McCormick varieties toward thiourea treatments is shown in fig. 6. The treated lots (lots *M* and *Q*, fig. 6) show complete failure of apical dominance, and also numerous examples of multiple sprouts per eye. At the time the treated tubers were photographed the check tubers had not sprouted (lots *E* and *F*, fig. 6), and the figure therefore shows the condition of the checks six weeks after the picture of the treated lot was taken. The Irish Cobblers show the strong apical dominance of tubers of this variety. This dominance was less marked in the case of the McCormick variety, but the effect of the thiourea on the number of sprouts per eye is clearly shown.

In many cases it was found that the apical buds of tubers treated with thiourea did not grow, but that growth first started from buds

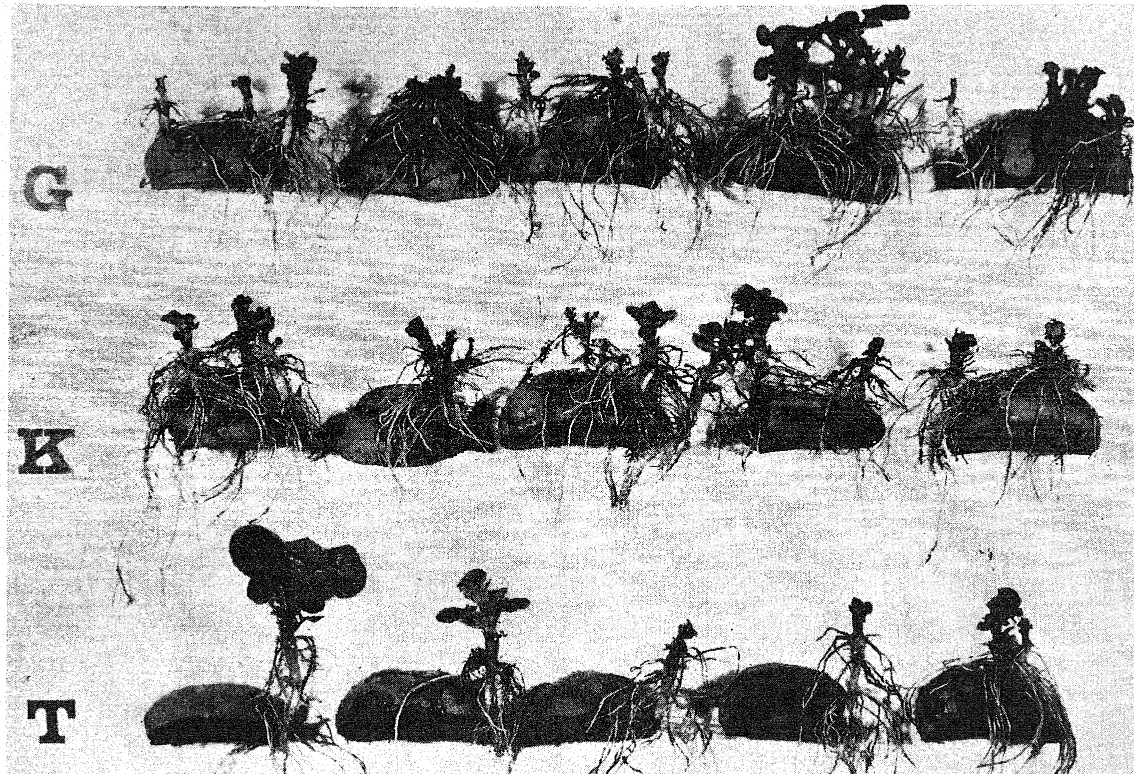


FIG. 5.—Lot G, tubers (Bliss Triumph) cut lengthwise into thirds and soaked two hours in 4 per cent thiourea; in each case apical end directed toward right; note absence of apical dominance and formation of multiple sprouts per eye. Lot K, same with 2 per cent thiourea for two hours. Lot T, check, soaked two hours in water; four out of five seed pieces show apical dominance, and three out of five show only one sprout per seed piece.

toward the basal end. This is shown in fig. 7 (top row, lot *D*), and the two pieces to the right in the second row (lot *H*). The failure

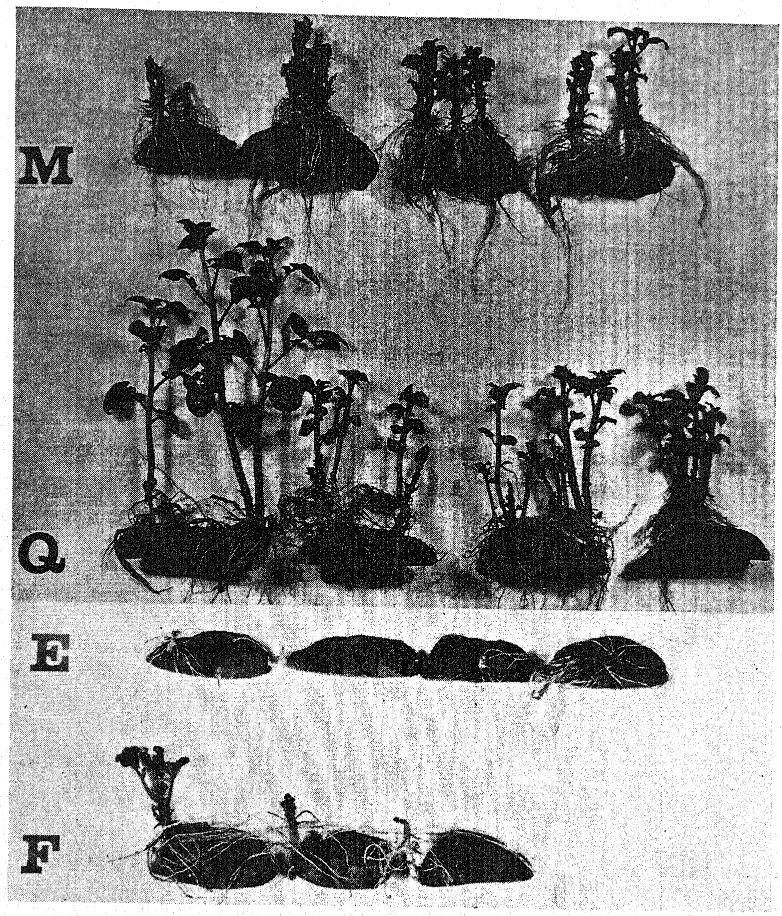


FIG. 6.—Lot *M*, tubers (Irish Cobbler) cut lengthwise into quarters, soaked two hours in 4 per cent thiourea; apical dominance disturbed and multiple sprouts per eye formed. Lot *Q*, same except McCormick variety used. Lot *E*, checks (McCormick) soaked two hours in water. Lot *F*, checks (Irish Cobbler) soaked two hours in water (lots *E* and *F* photographed 44 days after lots *M* and *Q*, but planted at same time).

of the apical buds to grow might have resulted from either of two causes: (1) the apical buds might have been injured or killed and

therefore incapable of growing; (2) the growth of the apical buds might have been inhibited by the growth of the basal buds that had started first.

That the first supposition is incorrect was proved by the fact that such apical buds, when cut off from the tuber and planted separately, sprouted at once. This is shown in fig. 7 (lot *K*, bottom row). The photograph was taken on the ninth day after the apical ends were removed and planted, showing that they had the capacity for prompt growth when removed from the inhibiting influence of the basal buds.

The results shown in fig. 7 indicate that by chemical treatment with thiourea the direction of dominance was reversed, a basally placed bud becoming dominant and inhibiting the growth of the apical bud. A reversal in the direction of dominance was also reported by Child and Bellamy (4) for *Phaseolus*. They found that non-apical buds, forced into growth as a result of physiological isolation from the tip, "in some cases may even inhibit the further growth of the chief tip." The situation here would be analogous if we assume that the thiourea treatment had caused physiological isolation of the apical buds. In what manner this isolation could be brought about by the treatment, however, is not known.

Discussion

The question whether this is a specific response to thiourea cannot be answered by these experiments. It can merely be stated that out of a list of more than 200 chemicals, representing various classes of chemical compounds, thiourea alone showed this effect upon tubers of these varieties. Further experiments would be necessary to determine whether by proper choice of concentration and time of treatment other chemicals could cause similar responses. While it seems unlikely that this is a specific response, it would be desirable to examine the possibility more closely; for if the number of chemicals that can influence bud dominance in this manner can be restricted to one, or only a few, or even to a certain group or type, the study of the cause of dominance will be much simplified.

Appleman (2) expressed the opinion that any treatment that weakened the sprouts destroyed the dominance of the apical bud.

Thus successive removal of sprouts and certain storage conditions resulted in loss of apical dominance. It is true that tubers treated

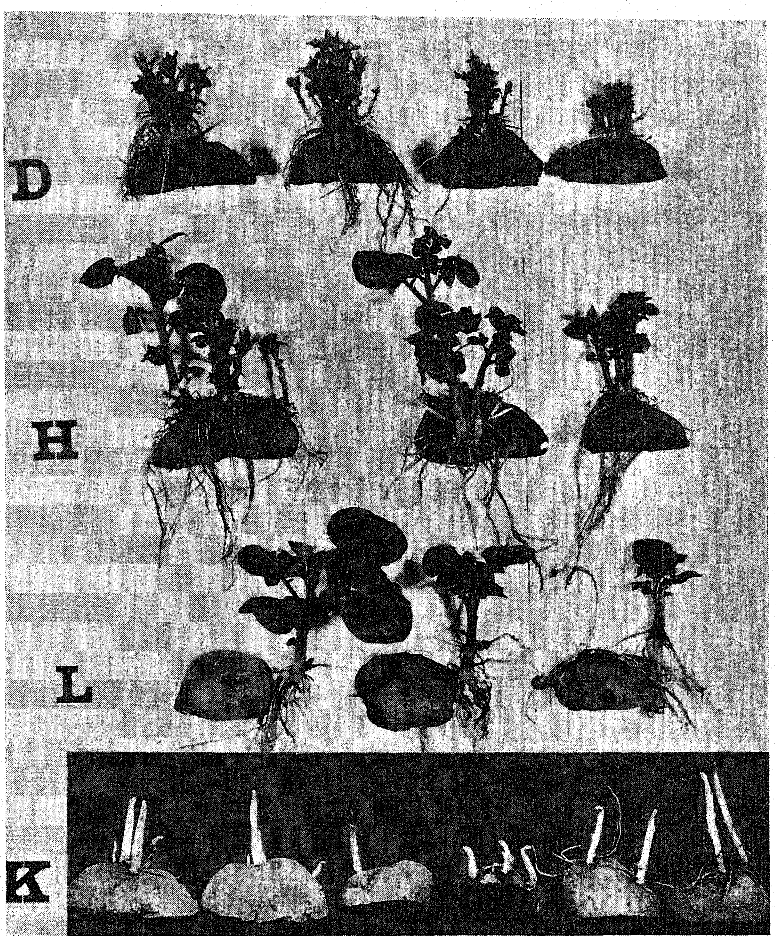


FIG. 7.—Lot *D*, tubers (Bliss Triumph) cut lengthwise into quarters, soaked one hour in 4 per cent thiourea; apical ends directed toward right; note apical buds were not growing, but buds more basally placed started into growth first. Lot *H*, same with 2 per cent thiourea for two hours; apical buds in the two pieces at right not growing. Lot *L*, checks, soaked two hours in water; only apical end growing and only one bud per eye started growth (compare lots *L* and *D*). Lot *K*, apical ends cut off from tubers like those shown in lot *D* and planted separately; inhibited apical buds started growth at once (photograph of lot *K* taken 9 days after planting).

with thiourea under conditions that forced many sprouts per eye did not give strong sprouts. They were thin and spindly. When six or seven were produced from one eye the sprouts were hardly stiff enough to stand upright. This may not be an expression of weakness, however; it may merely mean that there was insufficient food or other growth-promoting substances in the mother tuber to support so many sprouts.

From the standpoint of toxicity it is certain that thiourea is less toxic to potato than many of the substances used. Rotting of the treated pieces was common with most of the chemical treatments, but the thiourea treated lots were notably free from rot. Fig. 1 shows that the surfaces of the treated pieces were clean and free from discoloration or any other evidence of injury. Even when the treatment was severe enough to produce the result shown in fig. 2, the potato tissue was perfectly sound.

It is clear, therefore, that if this disturbance of the normal system of bud correlation in the potato is to be ascribed to a toxic effect, the injury must be caused in some special manner in the case of thiourea. It is believed that factors other than mere injury to the tissue are involved.

At least three theories have been offered to explain why the apical bud is able to prevent the growth of basal buds: (1) that the apical bud produces an inhibiting substance which travels in a basipetal direction and prevents the growth of buds lower down on the stem (ERRERA 6, REED and HALMA 10); (2) that there is a higher state of metabolic activity at the tip, and that, as a result of the electric potential set up by this physiological gradient, control is transmitted by way of the protoplasm and in some manner represses the growth of sub-apical buds, but not by the passage of any material substances (CHILD 5, CHILD and BELLAMY 4); (3) that not enough nutritive materials are available for all the buds present and that the growing tip causes a flow of materials toward itself and away from the basal buds (GOEBEL 8, GARDNER 7). Theories 1 and 3 are also fully discussed by MCCALLUM (9).

If inhibition is produced by a substance that is formed by the apical bud, the thiourea effect could be explained on the assumption that the treatment either prevents the apical bud from forming the

substance, or inactivates it in some way after it is formed. The fact that thiourea can nullify this inhibiting effect under certain definite conditions should be of great assistance in experimental work relating to the nature and mode of action of this hypothetical inhibitory chemical.

If the second supposition is correct, then the thiourea may operate, either by preventing the transmission of the stimulus, or by changing the relative metabolic activity of the apical and subapical buds. The results of the experiments, indeed, do show an increase in the rate of growth and therefore of metabolism in the basal buds, but there is little evidence yet to explain how thiourea can produce this change; and there is less to show how thiourea can cause the protoplasm to lose the capacity of transmitting the inhibitive stimulus.

There may be some evidence in favor of the third view, in that thiourea may contribute additional nutrient material by means of which additional buds may develop; but other nutritive substances were not able to induce this response. We would be compelled, therefore, to assume that additional amounts of only certain nutritive substances are necessary to initiate a renewal of growth in the inactive buds. We do not yet know how far the thiourea penetrates into the potato, nor what changes it may itself undergo after entrance into the tissue.

Practical considerations

When the forcing of multiple sprouts by solutions of thiourea was first noted, it was thought that from a practical standpoint this would be an unfavorable response; additional sprouts per seed piece would result in the formation of many small tubers per hill. It was pointed out to the writer by William Stuart of the United States Department of Agriculture, however, that when potatoes are produced for seed purposes and not for table use, a large proportion of moderately small tubers is desirable, since this size gives greater economy in planting a crop. A chemical treatment that would regulate with security the number of sprouts per hill, therefore, might find application. A further use for such a treatment might be found in the case of varieties that send out too few sprouts per seed piece.

The present high price of thiourea, however, about $15.00 per kilogram, would likely preclude its use in a practical way, and the possibility of using this chemical for such purposes will depend upon finding a cheaper supply of it. It may be that the high price is caused by the methods of purification, and that the impure or unrefined chemical would give equally good results in the potato treatments.

Summary

1. Solutions of thiourea (NH_2CSNH_2) caused the growth of two or more (often four or five) buds from a single eye of the potato.

2. This result was not caused by any other chemical at the concentrations and periods of treatment tried, although more than 200 chemicals representing various classes of chemical compounds were tested.

3. Several "urea" and "thio" compounds were tried, but none showed the consistent results that were obtained with thiourea. Next to thiourea, the most favorable chemicals for this purpose were the thiocyanates.

4. Solutions of thiourea also prevented the apical buds of the tubers from completely inhibiting the growth of basal buds. Certain cases were found in which the direction of dominance was reversed so that the apical buds themselves were inhibited. Such inhibited apical buds when cut off from the tuber and planted separately started into growth at once, after being removed from the influence of previously sprouted basal buds.

5. The relation of these facts to certain theories regarding the cause of the inhibition of basal buds by tip buds is discussed.

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CHEMICAL COMPOSITION OF ETIOLATED AND GREEN BERBERIS SPROUTS AND THEIR RESPECTIVE ROOTS[1]

E. R. Schulz and Noel F. Thompson

(WITH TWO FIGURES)

Introduction

The study of the behavior of *Berberis vulgaris* under different conditions was necessary in the investigations of means of eradicating this plant. As a consequence, certain facts were accumulated which bring out some points in regard to the metabolic processes taking place within the barberry. The results herewith reported, on the chemical composition of etiolated and green sprouts and their respective roots, were brought out by an experiment in which the effects of cutting off the tops of the barberry and the subsequent growth of sprouts from the roots were studied in relation to the rate of exhaustion of food material in the roots, and consequently of the death of the plant. In the two kinds of sprouts dealt with, it was noted that such plant constituents as starch, sugars, and ash differed markedly. As such facts dealing with the internal conditions are important to an understanding of the growth activity of plants, the data are presented in this paper.

The formative influence of continuous darkness and of shade on plants has frequently been studied. Weber (15) found in pea seedlings, on the basis of dry weight, more ash in green than in etiolated seedlings grown in a dark cellar. Palladin (5, 6) reported similar results in regard to ash in wheat and *Vicia Faba* leaves. He also found less protein in the etiolated than in the green leaves. Thatcher's (13) results with potatoes, peas, wheat, oats, and barley showed that shading caused a relative increase in moisture, mineral matter, and nitrogenous matter, and a decrease in carbohydrates. Stutzer and

[1] Cooperative investigation of the Wisconsin State Department of Agriculture, the University of Wisconsin, and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. The writers are indebted to H. B. Parmele, Agent, Bureau of Plant Industry, for some of the analytical work.

GOY (12) obtained higher ash content in shaded tobacco than in unshaded. SACHS (10) and others have shown that starch may be produced in aerial portions of plants even though these are kept in total darkness. That such darkened portions may store more starch than corresponding portions in the light does not seem to have been reported.

Experiments by SCHLOESING (8) indicated that the relative humidity of the atmosphere may be a very important factor. He grew some tobacco plants under glass jars and others without the jars, and obtained 19 per cent of starch in plants under jars, and 1 per cent of starch in plants grown in the open. He explained the high percentage of starch in the plants under the glass jars as being due to reduced transpiration rather than to reduced light. The bearing of these results upon the experiments reported in this paper will be discussed later.

Experimentation

MATERIAL

The analytical studies here recorded were conducted on four lots of *Berberis* tissues, namely, green sprouts and their roots, and etiolated sprouts and their roots. The plants used for the experiments (fig. 1) were growing in two localities with slightly different soil conditions, but approximately the same climatic conditions. The first locality was near Marshall, Dane County, Wisconsin. The soil was clay loam rich in humus, and the plants were large escaped bushes growing on the edge of a woodlot where birds and cattle evidently had dropped the seeds. Eight plants were selected at this place, four on May 19 and four on May 26, 1923. The tops were cut off close to the ground, and the stumps and roots of half of the number were covered with wooden boxes, lined on the inside with roofing paper to insure complete darkness; the other four stumps were left uncovered. Composite collections of roots from plants growing in the vicinity of the selected plants were taken at the beginning of the experiments. These samples were analyzed and used for subsequent comparison with identical storage products in the roots of the decapitated plants.

The second locality was near Gurnee, Lake County, Illinois. The soil here was a brown silt loam, very rich in humus. The bushes were

escaped from cultivation and were growing in a woodlot. The tops of 34 plants were cut off close to the ground on April 14, 1924, and over the stumps and roots of half of the number were constructed light-proof housings, 3 ft. square, made of stakes and roofing paper.

FIG. 1.—Type of *Berberis* plant used in these experiments.

The remaining 17 were left uncovered. A composite collection of roots from adjacent plants was collected and analyzed as was done at Marshall, Wisconsin.

The coverings at both Marshall and Gurnee proved capable of excluding light, as the developing sprouts showed no green; they

were always bright yellow, which is typical of *Berberis* and due to an alkaloid present in the tissues. Ventilation under the coverings was rather poor, but the growth of the sprouts under such cover was not less vigorous than in the open.

Methods

Collection and preparation of samples for analysis.—Several collections of sprouts and roots were made, as indicated in tables I–III. That portion of the samples intended for analysis in the dry state was always cut into small pieces, and within one hour of the time of collection it was given a preliminary drying for one-half hour at 98° C. to stop metabolic processes. The samples collected at Marshall were transported to Madison, where the laboratory was located, before giving them this preliminary drying. For the samples collected at Gurnee, an oven was provided near by where this drying was performed. Later, all the samples were dried at 65° C. for 10 hours, and then ground to a powder that passed through a 100-mesh sieve. From this ground material the carbohydrates were determined, since Link (3) found that they were altered in tissues dried at higher temperatures. For the moisture determination, a 2 gm. sample of this ground material was dried to constant weight at 102° C. The portion of the samples intended for analysis in the fresh state was carefully wrapped in oiled paper and transported to Madison as rapidly as possible. The material was then immediately chopped into small pieces and ground in a mortar with quartz sand and extracted with water, as described by Tottingham, Schulz, and Lepkovsky (14).

Analysis.—The Kjeldahl-Gunning method was used for nitrogen determination. Sugars and sugar equivalents of starch and hemicellulose were determined by the Shaffer-Hartmann (9) method. The sugars from the dried tissues were extracted with 90 per cent alcohol; the alcohol was then evaporated at 50°–55° C. under reduced pressure. From one fraction of this extract reducing sugars were determined, and the other fraction was hydrolyzed with 2.5 per cent HCl for 45 minutes previous to total sugar determination. Neutral lead acetate was used for clarification of the sugar-containing solutions. Saliva was used for removing starch from the sugar-free tis-

sue, preceding hydrolysis, and the starch was hydrolyzed by the action of 2.5 per cent HCl for 2.5 hours. The tissue from which the starch had been removed was hydrolyzed also with 2.5 per cent HCl for 2.5 hours, and the hydrolyzable fraction was designated as hemicellulose. Pentosans were determined from this hydrolyzable fraction, as well as from the residue left after the hydrolysis. The method of the Official Agricultural Chemists was used for pentosan determination.

The results of analysis, as shown in table II, indicate that the concentration of acid, and the duration of hydrolysis preceding total sugar determination were too great. The low total sugar content in the sprouts, which in 3 cases out of 12 is actually lower than the reducing sugars, must be due to the destruction of simple sugars such as levulose, which, according to BROWN and MORRIS (2), PARKIN (7), SPOEHR and MCGEE (11), and others, are found in abundance in the succulent parts of plants. The comparative ease with which the ketose sugars are affected by mineral acids also supports this assumption.

RESULTS

SAMPLES FROM MARSHALL, WISCONSIN.—Samples were collected on five different dates, as shown in table I. Each collection consisted of green and etiolated sprouts and the roots from which they grew. The percentages of moisture, ether extract, total sugar, starch, hemicellulose, and total nitrogen of each sample were determined. There were noticeable differences in the quantity of these constituents between the green and etiolated sprouts. Moisture and starch were lower in every case in the plants grown in the light; also nitrogen and sugar were lower in four cases out of the five. The ether extract and hemicellulose were the only substances determined that were approximately the same in the two sets of samples. With the roots, on the other hand, no definite differences could be determined, nor did the later collections show any appreciable decrease over the earlier ones.

SAMPLES FROM GURNEE, ILLINOIS.—The samples collected were similar to those from Marshall, Wisconsin, except that considerably greater numbers of plants were involved, and therefore they were more nearly representative. Additional substances also were deter-

mined in the analysis (tables II, III). The results agreed very closely with those of the former analyses. Only in the cases of ether extract and total nitrogen were there differences. In every case the former was higher in the green sprouts than in the etiolated ones, while the

TABLE I

ANALYSIS OF BERBERIS PLANTS GROWN IN LIGHT AND IN DARKNESS AT MARSHALL, WISCONSIN

PORTION OF PLANT	PLANTS GROWN IN	DATE OF COLLECTION	PERCENTAGE MOISTURE IN FRESH TISSUE	PERCENTAGE DRIED TISSUE				
				Ether extract	Total sugar	Starch	Hemi-cellulose	Total nitrogen
	Four plants cut down and half of the stumps covered May 19, 1923							
Sprouts	Light	June 18	81.00	6.66	3.49	3.81	13.65	2.58
	Dark	June 18	95.76	7.95	3.85	5.86	11.23	3.53
	Light	July 9	88.58	7.55	3.44	3.50	13.18	1.12
	Dark	July 9	96.20	4.60	8.31	6.40	10.78	2.45
Roots	Light	May 19	48.13	0.70	5.74	13.81	16.34	0.78
	Light	July 9	52.34	0.90	4.03	10.64	18.87	0.64
	Dark	July 9	54.70	1.06	3.63	16.28	15.88	0.73
	Four plants cut down and half of the stumps covered May 26, 1923							
Sprouts	Light	June 26	90.96	6.95	3.64	3.53	10.10	3.01
	Dark	June 26	94.91	7.43	4.70	4.37	11.48	2.77
	Light	July 19	87.09	6.03	1.76	2.60	13.75	2.28
	Dark	July 19	89.22	4.56	5.35	4.75	13.09	2.48
	Light	August 22	79.15	6.38	2.68	2.55	14.25	1.43*
	Dark	August 22	85.30	2.79	2.05	4.27	14.65	1.63*
Roots	Light	May 26	50.70	0.70	3.17	12.51	17.51	0.68
	Light	June 26	58.27	0.84	3.81	4.21	17.73	0.66
	Dark	June 26	56.65	0.79	3.99	4.35	17.35	0.65
	Light	July 19	56.49	1.52	3.23	6.00	19.36	0.72
	Dark	July 19	49.93	1.52	2.44	11.28	17.73	0.63

* Water-soluble nitrogen in the fresh sprouts was determined for the collection of August 22. The sprouts in the light had 0.47 per cent and in the dark 1.09 per cent, based on the dry weight.

nitrogen was about equally divided. Of the additional substances determined, reducing sugar (both from dried and fresh tissue), sucrose, water-soluble nitrogen, and ash were consistently higher in the sprouts grown in the dark than in those grown in the light. Pentosans were slightly higher in the green sprouts than in the etiolated ones.

At Gurnee, as at Marshall, there were no consistent differences in the substances found in the roots, nor was there an appreciable

TABLE II

ANALYSIS OF BERBERIS PLANTS GROWN IN LIGHT AND IN DARKNESS AT GURNEE, ILLINOIS

PLANTS GROWN IN	DATE OF COLLECTION	PERCENTAGE MOISTURE IN FRESH TISSUE	PERCENTAGE DRIED TISSUE							
			Ether extract	Sugar		Starch	Hemicellulose	Pentosans	Total nitrogen	Ash
				Reducing	Total					
			Sprouts							
Light	June 18	85.00	....	....	....	....	....	....	3.32	....
Dark	June 18	87.00	....	....	....	....	....	....	2.90	....
Light	July 2	84.36	2.83	2.16	3.02	4.07	11.30	5.20	3.12	5.65
Dark	July 2	87.47	1.25	4.52	5.72	4.62	8.95	2.58	3.32	9.90
Light	July 25	84.17	3.65	2.90	1.54	2.95	9.55	6.98	3.34	5.90
Dark	July 25	83.97	1.20	2.46	2.78	3.32	9.85	7.83	3.70	7.60
Light	August 16	80.57	2.85	2.46	2.58	2.15	10.85	7.43	2.67	5.40
Dark	August 16	84.65	1.05	3.20	3.90	2.95	9.55	7.38	3.03	10.20
Light	September 16	77.31	1.92	2.10	1.74	3.17	10.85	7.45	2.28	5.85
Dark	September 16	83.51	1.00	5.84	5.64	4.00	10.85	6.18	2.21	13.55
			Roots							
Light	April 14	55.00	0.38	1.30	3.70	9.95	11.75	8.97	0.82	9.50
Light	July 2	54.96	0.50	1.02	3.38	9.55	14.00	8.03	1.05	13.55
Dark	July 2	58.46	0.60	0.71	3.44	8.42	14.75	6.66	0.96	10.95
Light	July 25	56.51	0.27	1.54	2.72	10.05	13.85	8.42	0.94	7.05
Dark	July 25	55.83	0.07	1.24	2.28	6.70	15.20	9.28	0.86	5.45
Light	August 16	58.29	0.10	1.42	3.90	3.77	15.65	8.93	0.73	10.35
Dark	August 16	44.77	0.10	1.54	4.44	6.62	14.60	8.01	0.88	10.75
Light	September 16	53.27	0.00	1.54	3.15	7.07	16.55	8.87	0.75	4.25
Dark	September 16	52.97	0.03	1.54	3.60	8.12	15.80	8.20	0.80	5.70

lessening of the stored materials in the later collections over the earlier ones, even though a considerable quantity of sprouts had developed from them in the interval.

Discussion

A survey of the data presented indicates that sprouts from the roots of *Berberis vulgaris* differ markedly in chemical composition when grown in the light and under conditions which excluded light. In the latter case photosynthesis is absent, and growth and respiration of the sprouts are supported entirely by the translocation of products from the roots, which are rich in carbohydrates and rather rich in nitrogenous materials. Sprouts growing in the light, on the other hand, may draw upon the reserve materials stored in the roots as well as synthesize new materials. From this it would appear that the etiolated sprouts should be less abundantly supplied with carbohydrates than the green sprouts.

TABLE III

ANALYSIS OF WATER-SOLUBLE CONSTITUENTS OF FRESH TISSUE OF BERBERIS SPROUTS GROWN IN LIGHT AND IN DARKNESS AT GURNEE, ILLINOIS

Sprouts grown in	Date of collection	Percentage dried tissue			
		Reducing sugar	Sucrose	Total sugar	Nitrogen
Light	June 18	3.08	0.45	....	1.19
Dark	June 18	7.94	1.47	....	1.56
Light	July 2	3.30	0.41	....	1.26
Dark	July 2	6.70	1.90	....	1.67
Light	July 25	3.77	0.39	....	1.14
Dark	July 25	5.31	0.56	....	1.25
Light	August 16	1.51	0.40	2.42	0.70
Dark	August 16	5.23	0.78	6.24	1.33
Light	September 16	1.16	0.74	3.20	0.83
Dark	September 16	6.15	2.33	8.07	0.89

The results show, however, that carbohydrates, such as reducing sugars, total sugars, and starch were higher in etiolated than in green sprouts. Hemicellulose and pentosans were lower, as a rule, in the etiolated sprouts (fig. 2). These results, in this case from sprouts of woody plants, differ in some respects from results reported in the literature and obtained with plants grown from seeds. Various workers have reported the starch, or carbohydrate content

in general, as being lower in etiolated than in green plants. An exception is the work with tobacco reported by SCHLOESING and previously mentioned. High starch content was obtained in this case in plants with reduced transpiration caused by belljars. The tight housings made of roofing paper, under which the etiolated sprouts were grown, might have caused reduced transpiration, and thus resemble the belljars in the experiments of SCHLOESING. The question, however, of how reduced transpiration causes the accumulation of starch, if at all, is not clear and calls for further experimentation.

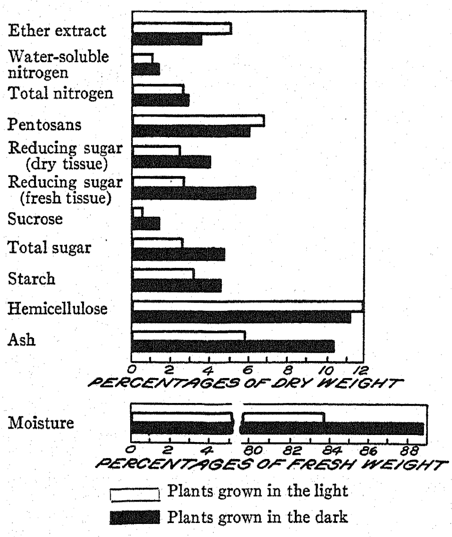

FIG. 2

FIG. 2.—Average percentages of all determinations of the various constituents of *Berberis* sprouts growing in light and in darkness.

More obvious, although not more definite, explanation in regard to high carbohydrate content in the etiolated sprouts is suggested by the growth habit of the plant under natural conditions. It is well known that it forms stolons which may serve as a storage place for excess nutritive materials. The condition of darkness might have changed the sprouts to stolons so far as composition is concerned. Experimental physiology supports this assumption in regard to the change of form, although no chemical composition of such forms is reported. PALLADIN (4) cites experiments in which above-ground tubers were developed on potato vines, and aerial rhizomes on stems of *Stachys palustris* and other plants by means of darkening a section of these plant parts.

The high content of soluble nitrogen found in etiolated sprouts (table III, fig. 2) is in agreement with the results of other investigations, such as those of SPOEHR and MCGEE. This might be due to amino acid accumulation when the plants were grown in darkness,

the amino acids acting as catalysts in increasing carbohydrate catabolism. Even if this were true, however, the carbohydrate translocation and storage were greater than the consumption.

The earlier workers found etiolated plants low in ash content. More recent investigations have shown etiolated or partly shaded plants higher in ash than plants grown in full light. The results with *Berberis* show the etiolated sprouts consistently higher in ash than the green, being as much as two and three times as high (table II, fig. 2). No satisfactory explanation of such behavior suggests itself. It may, however, be due to the etiolated plant having less stable compounds than the green, and these being capable of uniting with more inorganic constituents. The inorganic salts may also be a factor in aiding the metabolic processes in the absence of light, as BAUDISCH (1) has shown. He demonstrated that some salts, for example $Fe(OH)_2$, may cause oxidation, reduction, and synthesis of organic substances, thus in some respects taking the place of light in plant metabolism.

The roots of the etiolated and green sprouts, respectively, did not show marked differences or even appreciable decrease in nutritive storage materials due to translocation and utilization of such materials by the developing sprouts. The duration of the experiment was not sufficient markedly to affect the rich storage supply of the root.

STATE CAPITOL ANNEX
MADISON, WIS.

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PHYSIOLOGICAL INVESTIGATION OF BLACK HEART OF POTATO TUBER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 347

WARD B. DAVIS

(WITH SEVEN FIGURES)

Sometime prior to 1913, a blackening of the inner tissues of the potato during shipment was reported to experiment stations in northern United States. This condition, which was later found to be produced also in storage, is now known as black heart. Black heart is an abnormal discoloration of the central regions of the potato, in which there is a series of successive color changes from normal to pink, brown, and black on exposure to the air.

BARTHOLOMEW (6) made a preliminary report on black heart in 1913, and in 1914 (7) worked out the cause of the color change. No pathological organism was found. He believed that high temperatures maintained to prevent freezing during shipment caused increased respiration of the potato, and assumed that the increased respiration would naturally lead to an accumulation of carbon dioxide and a lack of oxygen in the central tissues. He thought that this accumulation of the products of respiration and lack of oxygen brought about the death of the cells, by which a greater amount of tyrosine was made available to be acted upon by the enzyme tyrosinase, to produce black melanic substances. Under laboratory conditions he produced the disease at 38°–44° C. in 15–24 hours. The optimum temperature for its development was 42°–44°.

Somewhat more recently, STEWART and MIX (24) have investigated the production of black heart, with the object of devising practical methods of preventing spoilage in shipping and storage. They showed that high temperature was not a necessary condition for producing the abnormality, since they produced it by confining the tubers in sealed jars for about 20 days at 12°–15° C. The conditions to which these tubers were subjected were similar to those resulting from deep piling and lack of ventilation. For this reason it

seemed probable that there were two important contributing factors in producing black heart in shipping and storage, high temperatures and lack of ventilation.

The present study was undertaken to determine some of the physiological changes which precede or accompany such profound changes in the cells of the interior. This was accomplished by producing the disease in the laboratory, and during its progress recording the following changes in (1) ratio of CO_2 to O_2 in intercellular spaces; (2) electrical conductivity of tissues; (3) catalase activity; (4) H-ion concentration.

Methods

In preliminary studies the methods later to be described were worked out separately. The individual variation of the potatoes in their susceptibility was so great that it soon became apparent that determinations made on the same tissue would more accurately indicate the changes involved. For this purpose, disease-free potatoes of the Rural New Yorker variety grown on peat soil were secured from a commercial firm in Minnesota. These potatoes were planted May 20, harvested September 20, pitted with dirt and straw until October 27, and then removed to a root cellar until the date of shipping, November 20, 1923. On their receipt at the laboratory they were stored at 17°–18° C., and the determinations were made in the ensuing three weeks.

Before these determinations were carried out, respiration of the normal tubers was determined. From 16 to 18 clean, well shaped potatoes were numbered, weighed, divided into two lots of approximately equal weight, and the respirations determined in the type of apparatus used by JOHNSTONE (12). The water bath used was electrically heated and regulated at 25° C. All results were reduced to standard conditions of 760 mm. pressure and 0° C. The respiration for each of the three series of tubers run was determined in the same way.

After the respiration had been determined, each lot of 8 or 9 tubers was placed in a 5 l. Pyrex desiccator provided with KOH, a thermometer, and two connections,—one to a graduated cylinder of pure oxygen, and the other to a manometer for keeping the pressure constant. The negative pressure developing in the oxygen cyl-

inder as the gas was withdrawn was constantly relieved by water from a water reserve to which the cylinder was attached. The type of water bath employed by MAGNESS and DIEHL (16) in apparatus for the determination of respiration of apples was used. The desiccator, oxygen cylinder, and water reserve were kept in an electrically heated and regulated oven at 45° C. Three series of two lots each, consisting of 16–18 potatoes to the series, were run. To avoid unusually large containers and to keep the tubers under as nearly the same conditions as possible, it was necessary to use the two lots to each series. Removal from the first lot began at the end of the first hour, while removal from the second lot was begun at the end of the eighth or ninth hour. Determinations were started immediately after removal.

GAS ANALYSIS.—By means of a cork borer, a sample 22 mm. in diameter was cut at right angles to the long axis of the potato, at about an equal distance from each end. Buds were avoided. From this sample the intercellular gases were extracted by means of an apparatus which MAGNESS (15) has described. The gases were collected over mercury, and analyzed for CO_2 and O_2 in the Bonnier-Mangin apparatus.

CONDUCTIVITY.—From the cylinder of tissue used for gas extraction, a second cylinder of smaller diameter was cut with a cork borer. About equal lengths were cut from the ends, so that the cylinder of interior tissue remaining was 13 mm. long and had a volume of approximately 1 cc. This cylinder was immediately placed between platinized platinum electrodes of a conductivity cell specially devised for this purpose (fig. 1). That the movable mercury electrode (*a*), which weighed 63.73 gm., always made good contact with the tissue was shown by the fact that the cylinder of tissue was often found adhering to it when it was lifted. The measurement of resistance was made in the usual manner, by making the conductivity cell one branch of a Wheatstone bridge. A Leeds and Northrup Kohl-

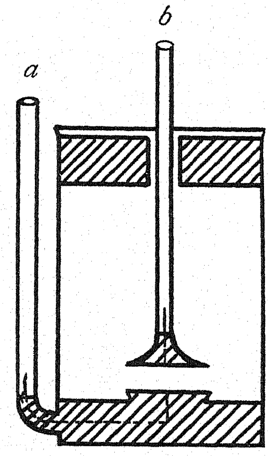

FIG. 1.—Conductivity cell

rausch circular slide wire bridge and a four dial resistance box were used. A microphone hummer, giving a thousand cycle frequency, was the source of the alternating current. Readings were taken at the middle of the bridge by balancing the known resistance against the unknown. Baldwin head phones were used for detecting the point of minimum sound. While the apparatus for determining resistance was quite accurate, the great differences in resistance between normal and abnormal tissue made unnecessary any attempt to secure the accuracy that can be obtained for solutions of electrolytes.

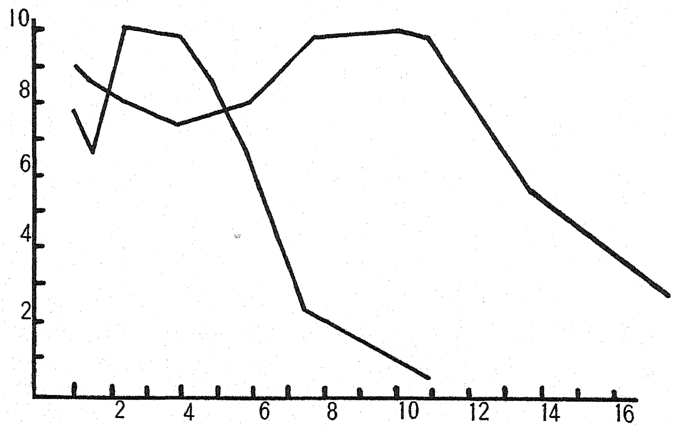

FIG. 2.—Conductivity of two potatoes (*A* and *B*) determined by inserting electrodes in each tuber separately; axes as in fig. 4.

Another method for getting a direct measurement of conductivity of the tissue was also used. While this method could not be used in the series just mentioned, it gave much more accurate indications of the course of the breakdown in a single tuber.

Two parallel pieces of steel 13 mm. apart and rigidly fastened together were thrust to the center of the potato from the stem end. Into the two slits thus formed two platinum foil electrodes with mercury connections were thrust, and sealed in with melted paraffin. The tuber was supported in a desiccator provided with a thermometer and a small opening to allow for the expansion of gases. The desiccator was then placed in an electrically heated and regu-

lated oven at 45° C. The electrodes were put in the circuit of the Wheatstone bridge in place of the conductivity cell, and readings made at will. Results obtained by this method are given in fig. 2.

CATALASE ACTIVITY.—As soon as the cylinder of tissue had been used for conductivity determinations, it was placed in a weighing bottle, weighed, and used for the determination of catalase activity. Essentially the same method as that which APPLEMAN (1) has described was followed. Calcium carbonate was used for neutralizing the acidity of the ground tissue and also of the "dioxogen." The amount of O_2 released from 10 cc. of standardized dioxogen in 10 minutes by 1 gm. of tissue was reduced to 0° C. and 760 mm. pressure.

HYDROGEN-ION CONCENTRATION CHANGES.—The portion of the tuber left after samples for gas analysis and conductivity were obtained was used for the determination of H-ion. CLARK and LUB indicators were applied directly to the tissues by a method similar to that described by ATKINS (4), PRIESTLY (18), and others. Since the indicators were applied before coloration of the tissues began there is no interfering color, and except for the vascular rings and buds which seem to be acid, the tissues are very uniform. Comparison of the injured with the uninjured areas was easily made in this way. Brom cresol purple and methyl red were used in each of the series of determinations. The former proved less satisfactory than the latter, especially at a P_H around 5.6–5.7.

TEMPERATURE.—The possibility that temperature effects, aside from causing increased respiratory activity, might be a factor, led to the use of the change in weight method which STILES and JØRGENSEN (25) have used to test the water-holding power of potato disks in relation to temperature. They found a critical point for water retentivity to lie somewhere between 30–40° C. Their methods were closely followed in determining this point a little more accurately for the potatoes used.

Data

PRELIMINARY STUDY.—An illustration of some of the results of preliminary conductivity studies is given in table I. This lot of potatoes, unconfined in jars, was placed in the oven at 45° C. The results show the variation in susceptibility due to size and other fac-

tors. Marked variation in resistance of the interior samples during the early stages of black heart is due to the impossibility of always including in the samples the affected tissues. In spite of this, even in preliminary studies where the conditions were not carefully controlled, the difference in conductivity between the interior and exterior tissues is quite marked.

TABLE I

CONDUCTIVITY OF INTERIOR AND EXTERIOR TISSUES OF POTATOES DURING PROGRESS OF BLACK HEART INJURY

No.	Weight in gm.	Loss in weight	Hours in oven	Ohms resistance		Condition of sample
				Exterior	Interior	
4...	110.00	....	0	6000	6000	Not colored
8...	105.60	....	3	5000	5000	Not colored
1...	132.10	0.43	6	6000	1800	Slightly colored
11...	142.65	0.47	10	7000	960	Slightly colored
			10	5700	1100	Slightly colored
2...	121.40	0.39	10	5300	450	Quite colored
			10	5000	1800	Slightly colored
			Average 10	5750	1078	
9...	182.93	0.68	13	6000	280	All colored
			13	5160	400	All colored
6...	140.37	0.59	13	5400	540	All colored
			13	4400	510	All colored
			Average 13	5240	433	
3...	167.20	0.800	16	3540	240	All colored
			16	5300	1160	Slightly colored
5...	142.18	1.780	16	4200	330	All colored
			16	6500	290	All colored
			Average 16	4885	505	
10...	171.85	1.03	19	5530	270	All colored
			19	4000	360	All colored
7...	132.75	0.95	19	5800	300	All colored
			19	4100	405	All colored
			Average 19	4882	334	

RESPIRATION.—The average results for the respiration of two of the three series run are given in table II. The determinations for the third series are not included, because of difficulties with the mechanism for temperature control of the water bath. Other tubers re-

served from the same lot did not sprout in storage at 17°–18° C. until two months later. They were partially after-ripened and heavily suberized at the time of the determinations.

TABLE II

RESPIRATION

	MG. CO_2 PER KG. PER HOUR	MG. O_2 PER KG. PER HOUR	CO_2/O_2
Average of four lots........	12.1217	9.9630	0.618

GAS ANALYSIS.—The ratios of CO_2 to O_2 in the intercellular spaces of the potato vary with the conditions of storage, such as temperature and depth of piling, and with the age of the tubers. The

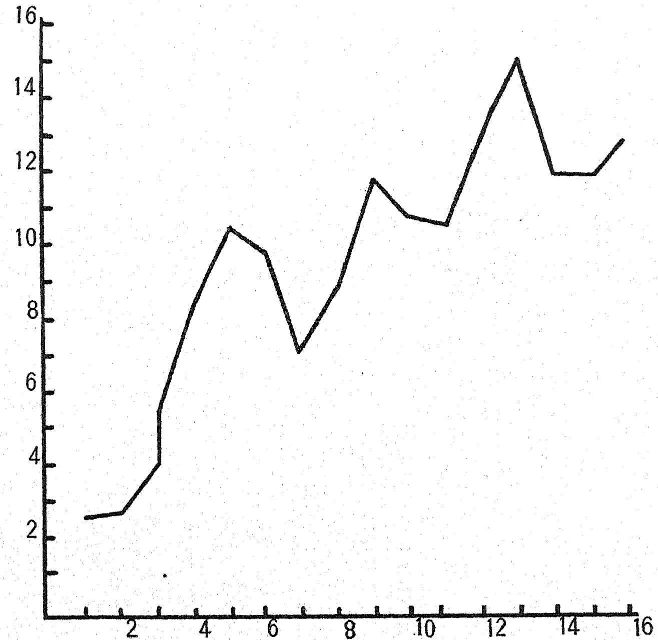

FIG. 3.—Ratio of CO_2/O_2: vertical axes represent ratio; horizontal axes represent time in hours.

amount of O_2 in the untreated tubers used in these experiments was 10–11 per cent, while that of the CO_2 was 5–6 per cent, making a ratio of much less than one. As shown in fig. 3, during the first hour

in the oven at 45° C. this ratio had increased to over two, in spite of the abundant O_2 in the atmosphere of the desiccator. Since the tubers removed from storage at 17°–18° C. cooled the interior of the desiccator somewhat, the temperature of the air surrounding the tubers was around 35°–36° at the beginning, and did not reach 45° until 5 or 6 hours later. At the end of 16 hours the percentage of CO_2 had increased to about 50, and the oxygen had correspondingly decreased to below 4 per cent. Since the samples from which the gases were extracted included a portion of the exterior as well as the interior tissues, the percentage of CO_2 in the interior must have reached far above 50.

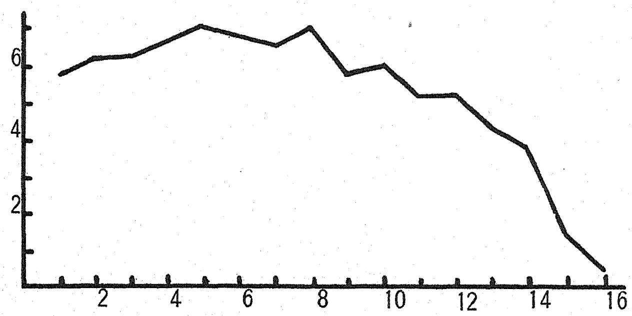

FIG. 4.—Average conductivity of three series: vertical axes represent resistance in thousands of ohms; horizontal axes represent time in hours.

CONDUCTIVITY.—OSTERHOUT'S (17) work on conductivity suggested the use of an accurate expression of the rate and degree of breakdown in the tuber during the progress of the disease. In spite of the variations already mentioned, the results of both the methods compare favorably in showing the course of the breakdown. The early rise of resistance which figs. 2 and 4 show took place at a period when the temperature of the potato must have been increasing, and when one would naturally expect the resistance to be falling. If tubers were cut open at the point where there is an unmistakable sharp fall in resistance and exposed to the air, the discolorations would appear in the central tissues. This fall no doubt is due to the increased permeability following the death of the cells in the region affected.

CATALASE ACTIVITY.—Before the problem was undertaken, the work of APPLEMAN (2, 3) had suggested a correlation between catalase and respiration. The ease with which catalase is determined makes this suggestion an important one. If catalase were correlated with respiration, the curve for catalase activity as given in fig. 5 should rise, at least at the beginning, but such is not the case. The curve for the decrease of O_2 in the intercellular spaces will more nearly parallel the curve for catalase activity.

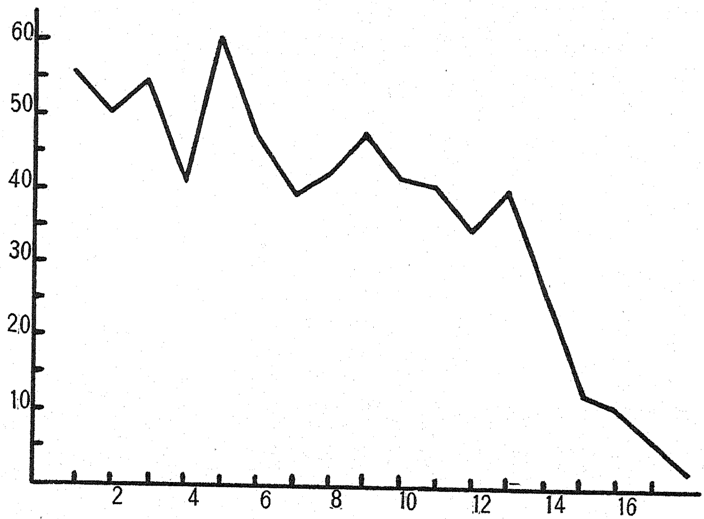

FIG. 5.—Catalase activity: vertical axes represent cc. of oxygen; horizontal axes represent time in hours.

HYDROGEN-ION CONCENTRATION CHANGES.—Although the H-ion concentration change revealed by the indicator method is small, there is an unmistakable increase, as indicated by fig. 6. Working under the best condition for detecting local changes, none were found. Within the limits of the method little importance can be attributed to the influence of the H-ion in causing localization of the affected tissues.

TEMPERATURE.—While it is by no means proved that heat is a factor in the production of black heart at high temperature, the abrupt change in the water-holding power of potato disks at about 38° C. (fig. 7) is quite suggestive. A slight loss of water with in-

creased permeability might be sufficient to hinder gaseous exchange by clogging the very small intercellular spaces. Very extensive clogging would hinder the extraction of gases, however, and this does

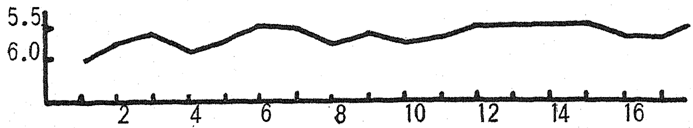

FIG. 6.—Hydrogen-ion concentration: vertical axes represent P_H value; horizontal axes represent time in hours.

not occur, except when tubers are left for much longer periods than those used in these experiments.

The pioneer work of DEVAUX (11) has left no doubt regarding the existence of intercellular spaces in many fleshy storage tissues, including the potato.

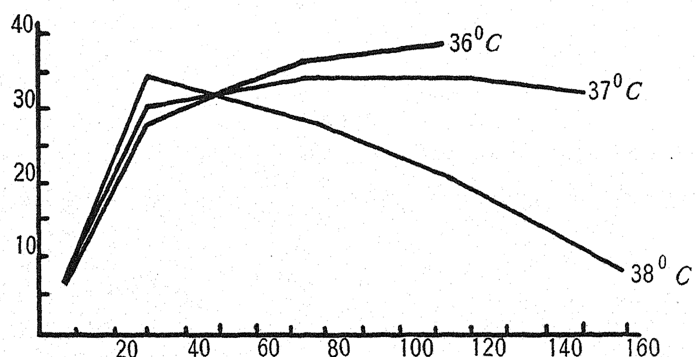

FIG. 7.—Water-holding power of potato disks: vertical axes represent percentage of water intake based on original weight of disks; horizontal axes represent time in minutes.

Discussion

The rapid accumulation of CO_2 to above 50 per cent, and the corresponding decrease of oxygen to below 4 per cent in 16 hours at 45° C., in a carbon dioxide free atmosphere, and with abundant O_2 supplied, has been shown in fig. 1. It is also quite likely that the percentage of CO_2 in that portion of the tuber which discolors on exposure to the air is much higher, and the O_2 lower than these re-

sults show. BARTHOLOMEW'S assumption concerning the accumulation of CO_2 is proved. Whether this is the cause of the death of the cells or not is not yet clear. STEWART and MIX, however, after finding that black heart is produced in a carbon dioxide free atmosphere, conclude that it is the lack of oxygen which is the cause of the death of the cells. BENNETT and BARTHOLOMEW (8) also claim support for this idea, stating that the accumulation of CO_2 seems to have no close relation to the production of black heart when potatoes are confined in jars. Unfortunately, however, they did not produce black heart in the absence of carbon dioxide within the tissues affected, nor were the relations of the internal gases found by analysis.

The results of other workers should be cited in this connection. PFEFFER (19) states that higher plants are injured by air containing 4–15 per cent CO_2, but believes that partial pressure rather than the percentage of CO_2 is important. CHAPIN (9) finds that CO_2 is toxic for plants in general. In their work with the apple, which possesses a tissue similar to that of the potato, MAGNESS and DIEHL (16) report that even when sufficient O_2 is present for oxidation, CO_2 accumulation as low as 19 per cent in the intercellular spaces inhibited respiration. Acidity under these conditions decreased. They suggest that since flavors resulting from such conditions are similar to those resulting from absence of oxygen, the action of oxidizing enzymes is inhibited, thus stopping normal oxidation. KIDD and WEST (14) have found that CO_2 is a factor in the production of brown heart of apples. Both of the latter workers (14, 16) analyzed the internal atmospheres of the apples used.

One of the most recent pieces of work that has to do with the relation of CO_2 and O_2 in animals is that of SAYERS and YANT (22), in which it is shown that a small amount of CO_2 mixed with the O_2 used for the resuscitation of persons affected with CO poisoning caused a quicker recovery than O_2 alone. This was probably due to the stimulative effect of CO_2. The term asphixiation, which BARTHOLOMEW has suggested, and which has been used in the most recent Department of Agriculture Bulletin (23) on potato diseases, is not very exact when applied to either plants or animals.

Figs. 2 and 4 illustrate the change in the conductivity. While it is quite possible that conductivity may be a measure of the permeabil-

ity of living tissue, it seems unwise to draw far reaching conclusions from the data at hand. The interpretations of OSTERHOUT (17), who takes the view that conductivity is a measure of permeability, is criticized by STILES and JØRGENSEN (26). They point out that increased conductivity may be brought about by changes which may not affect permeability. For instance, the breakdown of a complex, little ionized substance such as a protein to a simpler, more highly ionized compound such as an amino acid like tyrosine, may increase the conductivity without necessarily affecting the permeability of the tissues involved.

Conductivity, as determined either by the method in which cylinders of tissue were used, or by that in which the electrodes were inserted within the tuber, shows a rise before the fall in resistance. Only suggestions can be made as to the cause of the increased resistance at a period when one would expect it to fall. According to the views of CRILE (10) and OSTERHOUT, it might seem, for example, that the rise of resistance occurring just before death is just what we find as a natural phenomenon,—a sort of false recovery which often occurs shortly before death. OSTERHOUT finds that alkaloids and alcohols among other substances cause a rise before a fall of resistance in *Laminaria* tissues. It may be that alcohol or the alkaloid solanin is released in toxic concentration by the changes which take place in the potato tuber at high temperatures.

One might conclude that since confinement at low temperatures brings about the same breakdown as more rapid respiration at high temperatures, heat itself would not be a factor in producing black heart. Such may not be the case, however, since results of this study indicate that temperature becomes a critical factor in the retention of water by the potato tissues at about 38° C. It is still a question whether the greater amount of water in the inner tissues is sufficient to increase the respiration of those tissues, as the work of BABCOCK (5) suggests.

The curve of catalase activity falls during the period in the oven. Temperature is probably the main factor in this fall. Mrs. RHINE (21) found that catalase activity does not follow respiration in early stages of the germination of seeds of some cereals. LOEW's theory of catalase action is introduced to explain this difference. Since no lo-

calization of acidity was found, it seems unlikely that catalase activity was affected by acidity changes. RAPER and WORMALL (20), however, have produced evidence which suggests that acidity changes affect the rate of tyrosinase action and the nature of the pigment formed.

It is quite probable that the series of color changes taking place in black heart is the same as those occurring in connection with changes in the potato tuber produced by other means in which death occurs, although the name, black heart, has not been applied to the latter. Among methods producing such color changes may be mentioned killing with the electric current, infection with *Phytophthora erythroseptica* and other disease producing organisms, freezing injuries (JONES 13), and immersion in toluene or glycerine. In fact mere injury, such as crushing, produces the color change. Probably anything which causes the death of the tissues without destruction of the enzyme involved, or alteration of the nature of the substrate, may cause the same changes of color in the tissues killed.

Summary

1. This paper presents the results of a physiological study of black heart of the potato tuber.

2. Observations were made with the purpose of discovering the nature of the physiological changes which must precede or accompany the profound changes that take place in the interior region which suffers the breakdown and undergoes the color changes known as black heart.

3. The disease was produced in the laboratory at a temperature of 45° C., in a carbon dioxide free atmosphere in which abundant oxygen was available. The following determinations were made on the affected tissues over a period of about 16 hours: ratio of carbon dioxide to oxygen in the intercellular spaces, conductivity of the tissues, catalase activity, and H-ion concentration changes.

4. During the time preceding the incidence of black heart, carbon dioxide accumulates rapidly in the internal atmosphere and oxygen is rapidly depleted, until the intercellular gases contain more than 50 per cent of carbon dioxide and less than 4 per cent of oxygen.

5. This change in the internal environment of the cells is the first

change detected, and is followed by increasing permeability of the protoplasm, together with the other changes involved in the development of black heart. At the temperatures used, black heart is apparently the result of high respiratory activity and the failure of the gas exchange to keep pace with respiration rate, but the possible effects of temperature and other factors must not be overlooked.

6. The progress of injury to the protoplasm was followed by electrical conductivity methods, which enable one to judge the degree and rate of injury in any given set of conditions.

7. By these methods it has been shown that there is first a rise of resistance in the tissues, extending over a period which varies with the individual tuber at 45° C. This increased resistance is followed by a continuous fall, beginning with the death of the tissues.

8. These changes are more pronounced with the inner tissues of the potatoes. The cortical regions of the tubers show less change in resistance, and are also much slower to become affected with black heart.

9. The catalase activity of the affected tissues does not seem to be correlated with respiratory activity.

10. During the development of black heart there is a slight increase of H-ion concentration, but this change is not localized. The change as revealed by the indicator method is small and its significance not clear.

11. Temperature may exert a direct effect above 38° C., as some evidence has been obtained that this temperature becomes critical for the maintenance of the normal water relations of the cells.

12. Color changes similar to, or identical with those produced in black heart, may be induced in various ways. Probably anything which causes the death of the tissues without destruction of the enzyme involved, or alteration of the nature of its substrate, may cause the same changes of color in the tissues killed.

Grateful acknowledgment is made to Professor C. A. SHULL for suggesting the problem, and for help and criticism during the progress of the investigation, and also to Dr. S. V. EATON and Dr. G. K. K. LINK for suggestions made in regard to the work.

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LOS ANGELES, CALIF.

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BRIEFER ARTICLES

THE STRIP METHOD FOR SERIAL CELLOIDIN SECTIONS

(WITH ONE FIGURE)

During the past two years, the writer has been engaged in a survey of the vascular anatomy of the buds of a large number of gymnosperms and woody angiosperms. In dealing with such hard and resistant structures as winter buds, it was realized that paraffin would prove an unsatisfactory imbedding medium. Consequently the celloidin method was adopted and satisfactorily met the technical requirements of the problem.

Since the problem under investigation involved a study of the number and course of the bundles constituting the vascular supply of the bud scales and leaves, serial sections were absolutely necessary. Two of the methods recommended for serial celloidin sections were tested. The first method, in which various fixatives were employed in an attempt to fasten the sections on the slides, gave poor results, the majority of the sections dropping off in the staining process. In the second method, which requires the winding of a strip of tissue paper about the sections to hold them in serial arrangement during staining, the paper itself became so deeply colored that any accurate idea of the extent to which the sections were being stained was impossible. The following method was devised after considerable experimentation, and has given uniform results. It appears to be of enough general significance for anatomical work in celloidin to warrant a detailed account.

The material is imbedded in celloidin in the usual manner. When the thickening process has been completed, each bud, surrounded by a considerable quantity of celloidin, is removed from the bottle and molded into a ball-like mass before hardening in chloroform. Preparatory to sectioning, the celloidin ball is trimmed down to a conveniently small cube, an even margin being left on all four sides of the imbedded tissue. This cube is inserted directly in the clamp of the sliding microtome, without previously mounting it on a block of wood.

Each section as it is cut is removed from the knife with a brush and transferred to a clean slide kept moist with 70 per cent alcohol. The sections are placed serially on the slide in rows of five or six each, the size of the block determining the number. The writer has found that 10,

12, 15, or 18 sections are the most convenient numbers to handle on a slide; greater numbers are likely to cause trouble in the staining and mounting processes. When an appropriate number of sections has been cut and transferred in this way, a dissecting needle is used to arrange them on the slide so that their edges just touch without overlapping. The excess alcohol is now removed from the slide by means of a piece of filter paper, which is carefully pressed down upon the sections. Then, with a fine brush, which is kept lubricated in a small bottle of synthol, a 2 per cent solution of celloidin is quickly painted in between the sections. Fig. 1 shows the arrangement of the sections on the slide, the cross-hatching indicating the extent of the celloidin joints. The celloidin should not touch the tissue itself, but must be applied only to the points of contact of the sections. With a little experience, enough celloidin may be carried on the brush at one time to allow the necessary number of horizontal and

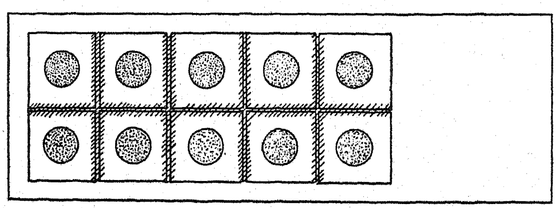

FIG. 1

vertical strokes to be painted, thus insuring smooth, even junctures between the sections. It is important to remove all the alcohol from the slide before applying the celloidin to the sections, as even a slight amount will cause the celloidin to harden on the brush and inevitably produce uneven joints.

The slide is now transferred to a rectangular staining box containing 70 per cent alcohol, to which a small quantity of chloroform has been added. Subsequent groups of sections are similarly treated and placed in the alcohol-chloroform bath until the desired series is complete. Thirty minutes in the bath proved sufficiently long to harden the joints between the sections.

The strips of sections now formed, if not already free from the slides, are removed by carefully inserting the edge of a section lifter between them and the slide while in the bath. A staining box containing 70 per cent alcohol, into which each slide may be separately transferred during this process, is necessary where a great number of strips is being handled. The strips are stored away in boxes of 70 per cent alcohol until

ready for staining. Properly prepared strips were found intact after a month in the alcohol, thus enabling a great number of sections to be prepared in advance of staining.

A number of practical suggestions are now offered in the hope that they may prove of value in other problems involving serial celloidin sections.

1. Thin, flat sections without wrinkles are desirable.

2. Rectangular staining boxes were used because of their adaptability to many phases of the process.

3. The strips should be thoroughly washed in distilled water before treatment with any staining reagents.

4. Chloroform must be added to the absolute alcohol used in destaining and dehydrating to prevent the dissolution of the joints between the sections.

5. In staining, a section lifter is employed in transferring the strips from dish to dish. The excess alcohol should be drained from a strip before transference by carefully touching it to a clean sheet of filter paper.

6. In the problem investigated, Haidenhain's iron-alum haematoxylin and safranin were employed as a double stain. Some difficulty was experienced in removing all the safranin from the celloidin matrix in the destaining process. It was found, however, that a light pink color left in the celloidin does not interfere with the optical properties of the preparation. The effect of other stains on the matrix has not been tested.

7. If thin, smooth junctures have been formed between the sections, the strips will lie fairly flat on the slides for mounting in balsam. Warping apparently is due to variation in thickness of the joints. Lead weights placed on the cover glass of the slides while they are drying are useful in smoothing out wrinkles in the strips.

8. Each strip constitutes a slide. In the anatomical problem investigated by the writer, sufficient changes in size and shape of parts permitted the slides to be arranged in serial order rather easily. In problems where topographical differences are less abrupt and conspicuous in the course of 10–18 sections, a small numbered paper tab may be glued with celloidin to the edge of each strip to indicate the sequence.—ADRIANCE S. FOSTER, *Bussey Institution, Boston, Mass.*

CURRENT LITERATURE

BOOK REVIEWS

Methods of descriptive systematic botany

Recently a new volume by HITCHCOCK has appeared.[1] This work is based upon its author's extensive taxonomic experience during the past 25 years. It contains some 220 pages, divided into 20 portions. Curiously enough, the author appears never to have seen DIELS's similar work[2] which was published four years earlier. This fact, however, is not without its compensations, for it affords the wholesome assurance that the book reflects the unfettered spontaneity and unswayed judgment of its author.

In the introduction, HITCHCOCK does not confine his efforts to describing the field to be treated. He presents also a discussion of "the scientific attitude." Seldom does one find, in so small a space, so able a treatment of this fundamental feature of all truly scientific work. Various chapters follow, upon categories in the natural system, nomenclature, authors of taxonomic groups, the use of manuals and floras, the identification of plants, etc. Generally speaking, the spirit of treatment throughout the volume is fair and impartial, giving evidence of a judicial temperament and restraint very much to be commended. In the main, the pitfalls of petty controversy have been successfully avoided. As further editions will doubtless be demanded, however, it may be appropriate here to offer certain minor suggestions and comments.

A considerable part of the book is devoted to three "kinds of taxonomic work leading to publication." These are "treated in detail as illustrating progressive steps in technique: the preparation of (1) a local flora; (2) a manual, or descriptive flora; and (3) a revision of a taxonomic group." In the reviewer's opinion, this sequence of the "progressive steps" is open to grave criticism. It is true, of course, that a prospective author should first have gained familiarity with many species in a given flora. But surely, before undertaking to write either a local or a general flora, he should have secured the perspective and judgment that come from intensive and protracted study of one or more small plant groups. In fact, many of the local floras are almost valueless for the very reason that their writer's training in taxonomy had emphasized breadth rather than depth. Again, in the chapter on the preparation of a local flora, we read:

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[1] HITCHCOCK, A. S., Methods of descriptive systematic botany. 1925.

[2] DIELS, LUDWIG, Die Methoden der Phytographie und der Systematik der Pflanzen (in Abderhalden, Emil, Handbuch der biologischen Arbeits methoden: Abt. XI. Methoden zur Erforschung der Leistungen des Pflanzenorganismus. Teil 1. Heft 2. pp. 67–190). 1921.

"On the basis of critical identification, the local botanist has an opportunity to work out the relationships of species in such a manner that his conclusions will form a valuable contribution to systematic botany." Still again, in the chapter on the preparation of a flora or manual, we read that the author of a flora should form his own judgment as to limits of genera and species. As a purely academic matter, it is probable that most taxonomists, and others too, would prefer to have the setting of these limits left largely in the hands of competent monographers.

The dictum that "the range of a species has no close connection with its abundance" is rather inadequately discussed. On p. 99 occurs the discouraging statement that "the species published since the preparation of the last supplement to the *Index Kewensis* must be found by a patient search through recent literature, especially abstracting journals." The student should be told that at Kew all new specific names are constantly being added to a large card index. In any case of importance, if the genera are known, a written request forwarded to Kew should secure any additional data promptly.

The pleasure derived by the taxonomist in reading the volume inspires the wish that a second edition might be similar in style but more extended in content. This is particularly true in the controversial field of herbaria and herbarium methods. It would have been commendable if HITCHCOCK had devoted more space to the vast importance of herbaria in the study of plant species. Especially does this seem true if one considers the state of neglect into which some of the world's almost priceless herbarium collections have been allowed to fall. At the risk of seeming dogmatic, he might well have taken a more positive stand in the matter of mounting specimens, instead of implying that either strapping or glueing alone was sufficient. Much irreparable damage has been suffered by herbarium specimens just because they had been only glued or only strapped. The chief objective in mounting (that is, the permanency of the specimen rather than, for instance, the beauty of its mounting) ought in the reviewer's opinion to have been emphasized. Furthermore, it is to be regretted that an authoritative set of conclusions by an impartial body of chemists, dealing with the moot but important question of effectively poisoning herbarium specimens, could not have been included. In some herbaria, almost entire families of plants have been ruined by insect ravages. Similarly, many of the common malpractices around a large herbarium appear to have been ignored completely: the use, on labels, of poor ink which fades after a few years; the use, for packets to hold often very valuable fragments, of cheap paper which soon crumbles away; the filing of photographs among the herbarium specimens instead of in separate albums, free from injurious chemicals, etc.

Viewing the book in its entirety, however, the careful reader cannot lay it aside without sensing its great importance as a pioneer work. It will doubtless exert for many years to come a dominant influence for good in its particular field.—E. E. SHERFF.

Mycetozoa

A third edition of LISTER's *Mycetozoa*[3] will be welcomed by students of this group. The first edition, published in 1894, increased interest in the subject and opened a wide correspondence which brought material from all parts of the world, and made a new edition desirable. This work was begun but LISTER died before it could be completed. His daughter, however, who had been constantly associated with him during the preparation of the first edition and the revision for the second, and who had painted many of the plates which are such an important feature of the work, continued the revision and made possible the second edition which appeared in 1911. In the third edition, three new genera and 46 new species are described, and 22 new plates have been added, 8 of which are colored. The keys are very complete and there is an extensive bibliography.

It is due to Miss LISTER's generosity that a book with 223 plates, 126 of which are colored, can be sold at a price within the reach of those who need the monograph.—C. J. CHAMBERLAIN.

NOTES FOR STUDENTS

Fluorescence of chlorophyll.—Our knowledge of the mechanism of photosynthesis increases slowly but steadily. In recent years it has been shown to be an adsorption process, and various workers have endeavored to solve some of its mysteries by the use of artificial systems with ultra-violet light. The chlorophyll itself, however, has received scant attention since WILLSTÄTTER's masterly researches made known its composition. NOACK[4] has attacked the problem from the pigment side, and has attempted to demonstrate that the fluorescence of the chlorophyll is definitely correlated with its photosynthetic activity. The thesis is not a novel one, for TSWETT advanced it fifteen years ago; but it is ably presented, with a quantity of interesting experimentation detailed in its behalf, and plentiful reference to the work of others which seems to support it. Unfortunately few but German citations are made. The questions are raised, whether illumination of fluorescing chlorophyll raises the oxidation potential of the system, and whether the energy change is of an order comparable with that of the work done in photosynthesis. Both these questions the author believes he can answer affirmatively.

The photo-oxidation catalyzed by fluorescing pigments was demonstrated qualitatively with benzidine, and quantitatively by the measurement of the consumption of O_2. Benzidine is readily oxidized on illumination in the presence of fluorescing dyes, such as eosin, in both aqueous and organic solvents, and as its brown oxidation product is readily detected, it proved very suitable for the

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[3] LISTER, ARTHUR, revised by LISTER, GULIELMA, A monograph of the mycetozoa, a descriptive catalogue of the species in the herbarium of the British Museum. 800 pp. xxxii+296. *pls.* 223. 56 wood cuts. British Museum, London. 1925. £1. 11s. 6.

[4] NOACK, K., Photochemische Wirkungen des Chlorophylls und ihre Bedeutung für die Kohlensäureassimilation. Zeitsch. Bot. **17**:481–548. 1925.

work in hand. Chlorophyll in true solution behaved like other fluorescing dyes, but its colloidal dispersion and solutions of Cu-chlorophyll, neither of which fluoresce, had no photo-oxidative effect. Furthermore, chlorophyll in both living and dead leaves caused photo-oxidation, and STERN has reported it as fluorescent in leaves, though WILLSTÄTTER had thought it colloidal.

Further evidence is found in a study of the bleaching of chlorophyll, a process which has often been shown to be an oxidation. The author finds that this bleaching is greatly delayed in the presence of benzidine, Na_2SO_3, the carotinoids, and other good acceptors of O^2, and offers the explanation of bleaching as an auto-photo-oxidation of the chlorophyll in the absence of better O_2-acceptors. Again the colloidal chlorophyll and the Cu-derivative are relatively photostable.

It is suggested that in photosynthesis CO_2 is the normal acceptor of the energy absorbed by chlorophyll on illumination. When the cell is deprived of CO_2, either directly or by traces of phenylurethan, which is preferentially adsorbed and so has the effect of excluding it, there may occur not only bleaching of the chlorophyll, but also an injury of the protoplasm by the well known photodynamic effect, first shown with bacteria by HERTEL. SO_2 has long been known to exercise a special toxicity for green cells, and it is found to have an effect similar to that of phenylurethan, so that an explanation of this toxic action is afforded on a photo-dynamic basis. In all these cases the chlorophyll absorbs radiant energy and must get rid of it again. In the absence of the normal acceptor, or of easily oxidized substitutes, the pigment and the protoplasm must be oxidized under the influence of the oxidation potential which has been built up.

The least satisfactory part of the paper is the theoretical portion, dealing with energy transformations. The author believes that WARBURG'S "primary photo-chemical product" is a peroxide, and has obtained in an artificial system the same results as WARBURG with green cells. In the absence of CO_2, the photo-energized chlorophyll molecule is believed to react with O_2, forming this peroxide, which then oxidizes the chlorophyll. The energy of this reaction is supposed to induce a chemi-luminescent fluorescence on the part of a second chlorophyll molecule. In the presence of CO_2, however, the energized chlorophyll is supposed to react with it, effecting its reduction, and again the energy of the reaction is called upon to cause the fluorescence of a second molecule of the chlorophyll. Here the primary peroxide formation is gracefully forgotten. As an alternative it is suggested, on the basis of some inorganic experiments of WIELAND, that the first step may be the activation of H_2 by transfer to it of energy from the chlorophyll. This activated H_2 may either react with CO_2, reducing it, or in absence of CO_2, with O_2 to form H_2O_2, which oxidizes the chlorophyll. Quite apart from the very involved mechanism of fluorescence, as here conceived, the theories encounter serious difficulties when the questions of actual electron and energy transfer are considered. The author does not enter into any details, but rather patches together theories with pieces extracted from the work of various investigators in other fields.

There are many instances in the work where the conclusions drawn are

based on insufficient evidence. The proof offered of a linear relation between O_2-consumption and O_2-partial pressure is open to question, because too few points were determined on the curve shown to assure that it really does not pass through the origin, as a straight line would not. Also, the demonstration of the equality of the energy change in illuminated chlorophyll and in photosynthetic work is possible only by considerable juggling of the few data obtained. Again, there is much stress laid on the fact that Cu-chlorophyll, which does not fluoresce, does not cause photo-oxidation. This observation would be much more valuable if it had been shown that the similarly derived Zn-chlorophyll, which does fluoresce strongly, was photo-oxidative, for the treatment producing these substituted phaeophytins may have produced intra-molecular changes.

In spite of these and other criticisms, however, the author has certainly shown that there is a definite relation between the fluorescence of chlorophyll and its photo-oxidative action, and he has given food for thought as to whether this relation may not be in some way of primary importance in photosynthesis, since the photo-oxidation is only the manifestation of an increased energy potential which can be diverted, perhaps, to photosynthetic use by CO_2. A new mode of attack has been suggested, and some problems already solved thereby. —H. S. Wolfe.

Island floras.—The great amount of endemism of many of the Pacific islands is a matter that has been known to botanists for years. The explanation of this phenomenon, and more particularly the accounting for the relationships existing between the floras of the different groups, presents many difficulties. Thus Juan Fernandez and Hawaii are volcanic islands remote from each other, but with rather closely related floras. Skottsberg has studied the former rather critically,[5] and has now made a careful comparison between the two.[6] He concludes that it is improbable that the vegetation has originated on the islands since they assumed their present shape, for they are young islands and the floras show evidence of considerable age. He presents evidence that seems to indicate that the Juan Fernandez flora, at least, is of continental origin, and that it existed long before the islands were formed. The flora seems to have gradually taken possession of the islands as the continent became submerged. A part of the flora has come from South America, thus explaining affinities with New Zealand and Polynesia. Hawaii also contains Antarctic types whose original home must have lain far to the south, and it seems probable that the history of the Hawaiian flora was similar to that of Juan Fernandez.—Geo. D. Fuller.

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[5] Skottsberg, C., Natural history of Juan Fernandez and Easter Island. **2**: pp. 238 Upsala. 1922.

[6] ———, Juan Fernandez and Hawaii: A phytogeographical discussion. Bernice P. Bishop Museum Bull. **16**:1–47. 1925.

Studies on Nematospora.—WINGARD[7] has published a valuable account of his investigation of *Nematospora Phaseoli*, one of the yeasts. It belongs to the group with needle-shaped ascospores, and was described originally as budding in the cotyledons of hazelnuts, producing asci from individual bud cells. The conclusions reached by WINGARD are briefly as follows. He finds that the genus is parasitic on a wide range of plants, but especially on the Leguminosae, and that infection is associated with the punctures of particular insects. Although the vegetative phase is usually yeastlike, in certain media it produces a rudimentary mycelium. In ascus formation it conforms to the usual Ascomycete program as to divisions and number of ascospores. The cytological details of nuclear division are also presented. As to relationships, the conclusion is reached that it is closely related to *Monospora* and *Coccidiascus*, so that these three genera form a natural group of Saccharomycetes.—J. M. C.

The flower of Euphorbia.—Miss HABER.[8] has attacked the difficult problem of the affinities of the Euphorbiaceae, which family "modern botanists place in the Geraniales." The previous conclusions have been drawn mostly from morphology and ontogeny, which Miss HABER has checked up with a study of the anatomy of the floral structures. The flowers of 33 species were studied, the specimens representing widely separated habitats. After presentation of the evidence, the conclusion is reached that the so-called "flower" represents an inflorescence in an advanced stage of reduction. The "involucre" comprises a whorl of alternate bracts and pairs of secondary branches of the inflorescence. The "ovary" is a pistillate flower terminating the main axis of the inflorescence. The process has been one of aggregation, suppression, and cohesion in the inflorescence. This certainly shows that the genus is very highly specialized, with extreme complexity and congestion and consequent reduction.—J. M. C.

Chemical bibliographies.—Valuable assistance to research is being rendered by the National Research Council through its publication of important bulletins. Attention is called to a recent bulletin compiled by WEST and BEROLZHEIMER,[9] for the Research Information Service of the Council. It consists of a bibliography of bibliographies on chemistry and chemical technology, covering the period from 1900 down to the close of 1924. It does for chemistry what previous bulletins have done for geology and physics. The first two sections list

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[7] WINGARD, S. A., Studies on the pathogenicity, morphology, and cytology of *Nematospora Phaseoli*. Bull. Torr. Bot. Club **52**:249–290. 1925.

[8] HABER, JULIA M., The anatomy and the morphology of the flower of *Euphorbia*. Ann. Bot. **39**:657–607. 1925.

[9] WEST, C. J., and BEROLZHEIMER, D.D., Bibliography of bibliographies on chemistry and chemical technology, 1900–1924. Nat. Res. Council Bull. 50. 8 vo. pp. 308. Washington. 1925.

the general bibliographies, and the abstract journals and year books. Part III lists the general indexes of serials, but the important section for chemists and biologists is section IV, which makes up almost the entire volume. It is an alphabetically arranged bibliography of special subjects. For instance, one finds almost a hundred bibliographies on metabolism, ten on hydrogen-ion concentration, about seventy on colloids, eighteen on photosynthesis, ten on respiration, etc. Of course the biologist will not use many of the lists, but these examples show how useful such a compendium may be to the investigator. There is a small group of personal bibliographies at the end of the volume.

The subject headings number about 2400, with about 10,000 bibliographies from 7500 authors. Naturally one could not expect a work of this kind to avoid omissions, but it will furnish a convenient starting point for investigation of the literature of the first twenty-five years of the century, so far as chemistry and chemical technology are concerned. The reviewer considers it a valuable addition to our source books of research.—C. A. SHULL.

Electrostatic theory of permeability.—About three years ago the writer[10] proposed an electrostatic theory of permeability based upon various results obtained in permeability studies previously published. This theory stated briefly that the effect of a salt depended: (1) upon the electrostatic charge on the colloidal elements or particles which make up the plasma membrane; and (2) upon the charges on ions of the penetrating salt, which must be considered individually. A cation with two positive charges is much more effective than two anions with single charges each. Antagonism was proposed as existing between cations and anions, and it was shown that, assuming that the protoplasm is negatively charged, the effect of a salt can be largely foretold in its effect upon the permeability of the protoplasm. In addition, most of the antagonistic effects reported up to that time were explained upon the basis of this theory.

In the last three years much additional work has been done by various investigators, and it is the purpose of this review to report the progress of the theory and to correlate with it as much as possible of recent important work, as well as to acknowledge contributions not previously mentioned.

In connection with the supposition that the protoplasm is negative in charge, it may be said that this rested upon the fact that the sea water and the blood plasma are both slightly alkaline, which would lead one to expect negative charges on the layers immediately in contact with these fluids. Further, the cataphoresis of blood corpuscles toward the positive pole indicated the presence of a negative charge.

Of the work done since the writer's paper appeared, of prime importance is that of HEILBRUNN,[11] who has brought evidence to show that the interior of the

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[10] RABER, O., Permeability of the cell to electrolytes. BOT. GAZ. **75**:298–308. 1923.

[11] HEILBRUNN, L. V., The colloid chemistry of protoplasm. I. General considerations. II. The electrical charges of protoplasm. Amer. Jour. Phys. **64**:481–498. 1923.

———, The electrical charge of living cells. Science **61**:236–237. 1925.

cell is charged positively and the exterior negatively. This has been concluded from the measurement of changes in the fluidity of the internal protoplasm. Ca and Mg ions make the inner surface more fluid than monovalent ions, while Ce and Al are still more active. Thus cations have the same loosening effect upon the inner protoplasma which anions have upon the outer (as found in *Laminaria*). HEILBRUNN supports the electrostatic theory completely, and agrees that the antagonistic action of salts is to be explained by their effects upon the viscosity of protoplasm. He explains the difference in sign of the outer and inner protoplasm as due to the rate of diffusion from the surface of HCO_3 and CO_3 ions, which he thinks would account for the difference in electrical charges. This simply means that the interior of the cell is acid because it is in contact with the products given off by catabolism which result in acid solutions. The exterior is alkaline because it is in contact with an alkaline solution. Most determinations of the P_H of the cell sap show it to be acid, which supports this hypothesis.

Another interesting support comes from the work of BROOKS,[12] who finds that pentavalent arsenic penetrates the protoplasm and goes through into the cell sap of *Valonia* less easily than the trivalent form. This is comparable with the writer's work on iron in its bivalent and trivalent forms.

More recent work has been reported by SCHAEDE,[13] who finds by the use of stains that the living protoplasm in the onion is basic, while dead protoplasm and cell sap have an acid reaction. He suggests that the dead protoplasm may not be acid in itself, but is made so by the acid cell sap.

In the work on antagonism and permeability many papers support the electrostatic hypothesis. As examples of these only a few of the more important will be cited.

An interesting paper in this connection is that of FREE,[14] which, although published before, did not come to my attention until after my theory had been published. He suggests that the state of aggregation of the particles of the protoplasmic membrane is the determining factor in permeability. He does not suggest that electrostatic effects are of prime importance in bringing about these changes, but the paper is mentioned here so that the literature may be complete.

A more suggestive paper by GREEN[15] appeared about the same time. He worked with gelatin, and suggested from the results obtained that balanced solutions were important in plant growth because they were in equilibrium with iso-electric protein. We see now that they are in equilibrium, not with iso-

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[12] BROOKS, M. M., The penetration of trivalent and pentavalent arsenic into living and dead cells. Proc. Soc. Exp. Biol. and Med. 21:50–51. 1923.

[13] SCHAEDE, R., Über die reaktion des lebendes Plasmas. Ber. Deutsch. Bot. Gesells. 42:219–223. 1924.

[14] FREE, E. E., A colloidal hypothesis of protoplasmic permeability. Plant World 21:141–150. 1918.

[15] GREEN, N. B., The effect of ions of NaCl and $CaCl_2$ upon the electrical conductivity of certain colloidal mixtures. Plant World 21:303–316. 1918.

electric protein, but with protoplasm which is negative in charge; but GREEN was correct in connecting permeability with electrostatic charges. This paper of GREEN'S is connected with previous work of FENN,[16] to whom GREEN expresses his indebtedness. FENN had studied the precipitation of gelatin by alcohol and the antagonistic effect of salts upon this process. He saw the importance of electric charges in this connection, and accepted the electrostatic explanation of oppositely charged ions as the simplest one for the antagonistic effects observed.

The writer's hypothesis has explained the ordinary antagonism between NaCl and $CaCl_2$, but examples are constantly recurring in the literature, such as that reported by WINSLOW and FALK[17] for *Bacterium coli*. In dilute solutions of five parts NaCl and one of $CaCl_2$, not only is all toxicity destroyed but there is even a stimulating effect observed.

CHOLODNY[18] finds that electrolytes increase the viscosity of protoplasm, which he attributes to the action of the cation on the negatively charged disperse phase. Polyvalent anions inhibited this cation action.

SCARTH[19] finds that the permeability effects in *Spirogyra* depend upon the valency of the ion. The method he used for determining penetration was one which measured *ultimate* penetration, and does not separate it from the first effect, which may be a decrease of permeability. The paper is very suggestive, however, and can easily be interpreted as a support for the electrostatic hypothesis.

By far the most important work in this field has been done by KAHO,[20] who was publishing at the same time as the writer. KAHO'S summary (condensed) is as follows:

"1. The penetration of neutral salts into plant protoplasm is a physicochemical process, which depends upon the colloidal activity of the salts in which both ions are important.

"2. The penetration of the salt depends upon its ability to change the colloidal conditions of the plasma membrane, in which the action of each salt is

[16] FENN, W. O., Similarity in the behavior of protoplasm and gelatin. Proc. Nat. Acad. Sci. **2**:539–541. 1916.

[17] WINSLOW, C. E. A., and FALK, I. S., Studies on salt action. IX. The additive and the antagonistic effects of sodium and calcium chlorides upon the viability of *Bact. coli*. Jour. Bact. **8**:237–244. 1923.

[18] CHOLODNY, N., Über Protoplasmaveränderungen bei Plasmolyse. Biochem. Zeitsch. **147**:22–29. 1924.

[19] SCARTH, G. W., The penetration of cations into living protoplasm. Amer. Jour. Bot. **12**:133–148. 1925.

[20] KAHO, H., Ein Betrag zur Permeabilität des Pflanzenplasmas für die Neutralsalze IV. Biochem. Zeitsch. **123**:284–303. 1921.

———, Über die physiologische Wirkung der Neutralsalze auf das Pflanzenplasma. Bull. no. 18, Bot. Inst. Dorpat Univ. 1–167. 1923.

the additive effect of the oppositely acting ions. The kations have a coagulating effect which lowers the permeability for the salt. The anions have a peptising or dissolving action on the plasma colloids, which increases the permeability of the salt. The resulting action of a neutral salt is the algebraic sum of these opposite tendencies.

"3. The toxicity of a salt is the result of its penetrability.

"4. The lipoid materials of the surface layers of the plasma play an important part in the intake of neutral salts, thus supporting the work of HANSTEEN-CRANNER[21]."

KAHO'S work and my own have both stressed the fact that antagonism is between cations and anions rather than between cations alone, as had been commonly held formerly, but KAHO does not emphasize electrostatic effects quite so much or give a definite picture as to how these electrostatic differences are effective.

KAKIUCHI,[22] in work done on the precipitation of phospholipin, obtains results similar to KAHO and the writer. He agrees that the antagonism is between cations and anions, but holds that the lipoids of the plasma membrane are the responsible colloids, in which he may be correct. The electrostatic hypothesis as formulated by the writer did not deal with this aspect of the matter. In case the lipins are the materials of the membrane which are affected by the salts, many of the phenomena observed by OVERTON could be correlated still further than when the electrostatic hypothesis was proposed. The findings of KAKIUCHI and HANSTEEN-CRANNER, in fact, link up the electrostatic theory with the lipoid theory in a very interesting and suggestive manner.

MANN[23] finds that basic dyes are absorbed more than acid by mangold tissue, that is, that the tissue is more permeable to basic dye, as might be predicted. The dye intake is antagonized by salts and the antagonism is a function of the valency of the cation, just as the intake of acid or positive dyes is antagonized by anions, and varies with their valency. This work thus supports the colloid precipitation theory, and the electrostatic theory of permeability.

MACDOUGAL,[24] [25] who has worked extensively on the penetration of salts into succulent tissues and their effects upon permeability, has taken a decided stand for a colloidal, electrostatic explanation of permeability phenomena, and

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[21] HANSTEEN-CRANNER, B., Beitrage zur Biochemie und Physiologie der Zellwand und der plasmatischen Grenzschichten. Ber. Deutsch. Bot. Gesells. **37**:380–391. 1919.

[22] KAKIUCHI, S., Studies on physico-chemical properties of phospholipin. Jour. Biochem. Tokyo **1**:165–174. 1922.

[23] MANN, C. E. T., The antagonism between dyes and inorganic salts in their absorption by storage tissue. Ann. Botany **38**:753–777. 1924.

[24] MACDOUGAL, D. T., Permeability and the increase of volume of contents of living and artificial cells. Proc. Amer. Phil. Soc. **62**:1–25. 1923.

[25] ———, Growth and permeability. Carn. Inst. Year Book **23**:125–131. 1924.

says: "The most useful explanation which has as yet been offered in this matter is that of RABER, in which these effects are taken to rest upon the relative density of the charges upon the kations and anions acting upon the colloids."

Many other articles from recent literature could be cited, but these are sufficient to show the kind of support now given to the electrostatic theory from various angles, and to show that this hypothesis gives promise of extreme utility, whether the colloid particles concerned are lipoid, protein, or both.—O. RABER.

Taxonomic notes.—REHDER[26] has published some results of his investigation of the material assembled at the Arnold Arboretum. The result is a description of 1 new species, 8 new varieties, 17 new combinations, 17 new forms, and 4 new names of shrubs and trees.

WILSON[27] has published a detailed account of the Taxaceae and Pinaceae of the Yunnan province of China, from material collected by J. F. ROCK under the auspices of the National Geographic Society. The Taxaceae are represented by 8 species, belonging to *Taxus*, *Podocarpus* (3 spp.), *Torreya*, and *Cephalotaxus* (3 spp.). The Pinaceae include 33 species in 15 genera, Yunnan being richer in this group than any other region of China, and also including 3 interesting endemic species.

WELCH[28] has published a monograph of the North American representatives of *Cucurbitaria*. He recognizes 25 species, and excludes 43 species previously listed under the genus.

BURT,[29] in continuation of his investigation of the Thelephoraceae of North America, has published a very detailed account of the genus *Peniopora*. Full record is made of distribution and of material examined. He recognizes 120 species, 67 of which are described as new. There are also 11 new combinations.—J. M. C.

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26 REHDER, ALFRED, New species, varieties, and combinations from the herbarium and the collections of the Arnold Arboretum. Jour. Arnold Arboretum **7**:22–37. 1926.

27 WILSON, E. H., The taxads and conifers of Yunnan. Jour. Arnold Arboretum **7**:37–68. 1926.

28 WELCH, D. S., A monographic study of the genus *Cucurbitaria* in North America. Mycologia **18**:51–86. 1926.

29 BURT, E. A., The Thelephoraceae of North America. XIV. Ann. Mo. Bot. Gard. **12**:213–357. 1925.

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RELATION OF HYDROGEN-ION CONCENTRATION TO GROWTH OF CHLORELLA AND TO THE AVAILABILITY OF IRON[1]

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(WITH FIVE FIGURES)

Introduction

In recent years there has accumulated considerable experimental evidence indicating that the growth of the higher green plants, as well as of fungi and bacteria, is influenced by the H-ion concentration of the culture medium. This has been demonstrated particularly in the case of some of the crop plants, where, of course, the results may prove to be of much practical importance. Field and pot experiments with higher plants, however, are always complicated by the presence of microorganisms in the soil, and by the difficulty of maintaining a constant H-ion concentration in the medium. Even when pure culture technique is employed, the complex structure of the plants used as experimental material renders a correct interpretation of the results difficult. Moreover, in many cases there is considerable uncertainty as to whether the effect produced by varying the reaction of the medium is one of H-ion concentration per se, or of the unavailability, as the result of precipitation, of some essential mineral element or elements of the nutrient solution. It occurred to the writers that the use of a unicellular green alga, grown in a nutrient solution of high buffer content, might simplify some of these

[1] This work was done at Cornell University under fellowships in the biological sciences, National Research Council. The writers wish to express their appreciation to both these institutions for the facilities which made the investigation possible.

difficulties and make possible a more exact analysis of the effect of the H-ion concentration on the rate of growth of a green plant.

The organism used in these experiments was a large celled species of *Chlorella*, of uncertain identity. This alga was obtained originally from soil, was isolated in pure culture, that is, one free from all other organisms, and for a number of years has been cultured in this laboratory on agar slopes.

Methods

The procedure adopted was to introduce uniform amounts of the pure culture of *Chlorella* into liquid culture solutions in Pyrex Erlenmeyer flasks of 150 cc. capacity. For each culture, 50 cc. of a mineral nutrient solution was supplied. Unless otherwise stated, the salt content of the solutions was the same in all cultures except for the varying amounts of the phosphate buffers used to secure the range of P_H desired. The dry weight of the crop produced after a definite time interval was used as a criterion of growth. It is realized that for certain types of solutions, the dry weights of the crops do not furnish a fair basis for growth comparison in *Chlorella*, due to the unequal starch content of the cells. Microscopic examination of the individual cultures of these experiments, however, did not show noticeable differences in this respect.

Experiment 1: Growth in complete nutrient solution at varying H-ion concentrations

The purpose of the first experiment was to determine the possible acid and alkaline limits for growth, as well as H-ion concentrations favorable for growth. For the cultures of this series a nutrient solution, designated as solution *A*, was prepared as follows:

$Ca(NO_3)_2 \cdot 4H_2O$	2.95 gm.
$MgSO_4 \cdot 7H_2O$	0.4 gm.
$Fe_2(SO_4)_3$	Trace
Glucose	20 gm.
Distilled water to	1000 cc.

Phosphate buffer mixtures, designated as solution *B*, were prepared from the following phosphate solutions:

M/7.5 KH_2PO_4	18.156 gm. per liter
M/7.5 K_2HPO_4	23.23 gm. per liter
M/7.5 H_3PO_4	13.07 gm. per liter

Various mixtures of these phosphate solutions were prepared, designed to give a range of H-ion concentration from P_H 2.8 to 7.13. Twenty-five cc. of solution *A* was introduced into each of a number of Erlenmeyer flasks and sterilized. To obtain the complete culture solutions of the desired H-ion concentrations, each of these portions of solution *A* was combined with a similar amount of sterilized solution *B*. This combination was effected with the usual aseptic precautions after both solutions were cool. The separate sterilization of solutions *A* and *B* prevented the caramelization of the sugar in the

TABLE I

COMPOSITION AND INITIAL P_H OF CULTURE SOLUTIONS FOR EXPERIMENT 1

No.	SOLUTION *A* (cc.)	M/7.5 H_3PO_4 (cc.)	M/7.5 KH_2PO_4 (cc.)	M/7.5 K_2HPO_4 (cc.)	INITIAL P_H ELECTROMETRIC	INITIAL P_H COLORIMETRIC
1..........	25	3.75	21.250	....	2.81	....
2..........	25	2.50	22.500	....	3.07	3.30
3..........	25	2.00	23.000	....	3.20	3.40
4..........	25	1.00	24.000	....	3.47	3.60
5..........	25	....	25.000	....	4.14	4.30
6..........	25	....	24.375	0.625	4.62	4.70
7..........	25	....	23.750	1.250	4.94	4.95
8..........	25	....	22.500	2.500	4.82(?)	5.05
9..........	25	....	17.500	7.500	5.73	5.80
10..........	25	....	10.000	15.000	6.31	6.30
11..........	25	....	5.000	20.000	6.68	6.60
12..........	25	....	1.250	23.750	6.95	6.90
13..........	25	....	0.250	24.750	7.10	7.00
14..........	25	....	....	25.000	7.13	7.00

alkaline cultures, and to some extent the precipitation of magnesium and calcium phosphates. It will be noted that in the completed culture solutions the concentrations of salts in solutions *A* and *B* were decreased one-half.

Four replications of each H-ion concentration were prepared; one was used for the initial P_H determinations, and the remaining three in each case were inoculated. The H-ion determinations were made electrometrically and checked, except in the case of the most acid solution, colorimetrically, using GILLESPIE's (4) drop ratio method. A bubbling type of electrode was found satisfactory in making the electrometric determinations. The apparatus was checked frequently, using an M/20 potassium hydrogen phthalate buffer mixture, as recommended by CLARK (1). The composition of the complete cul-

ture solutions and the data for the initial H-ion concentrations are given in table I. The initial P_H is also presented graphically in fig. 1.

INOCULATION.—The organism, *Chlorella* sp., was cultured on agar slants, and from these a suspension was prepared by scraping the cells from the surface of each slant and adding them to a sterilized balanced solution. This solution, which was used to prevent

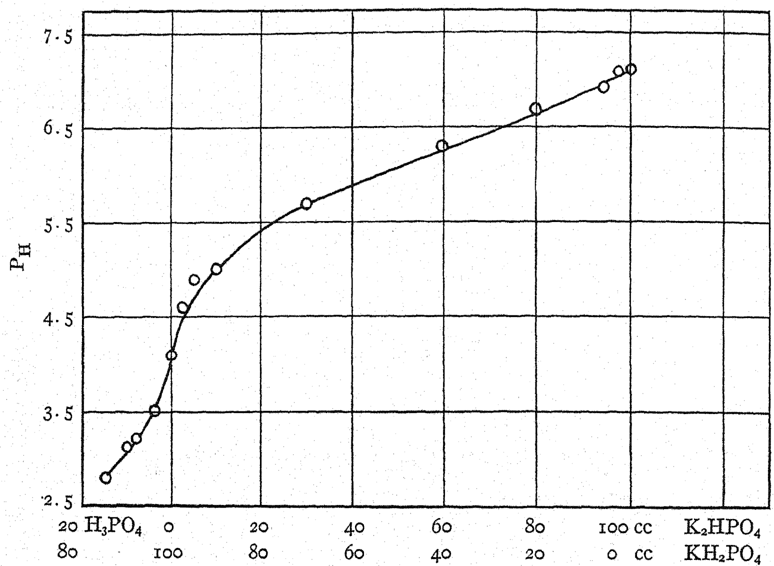

FIG. 1.—Composition-P_H curve of culture solutions used in Experiment 1: values of abscissae represent cc. of each constituent of buffer mixtures per 200 cc. of culture solution.

injury to the cells, was made up according to the method of OSTERHOUT (6), by mixing 95 cc. of a 0.6 per cent NaCl solution and 5 cc. of a 2.2 per cent solution of $CaCl_2$. To each culture flask to be inoculated, 0.5 cc. of this suspension was added, observing the usual aseptic precautions. The suspension was thoroughly agitated before taking sample. A microscopical examination of the algal cells in the balanced solution was made on the day following the inoculations, and they appeared to be normal.

The solutions were inoculated on April 15, 1924, and were placed at once on a shelf at the north window, where they were all quite

evenly lighted but never in direct sunlight. The first growth observations were noted down on April 24, nine days after inoculation. At this time considerable growth was observed in those solutions with

TABLE II

OBSERVATIONS NINE DAYS AFTER INOCULATING IN SOLUTIONS OF VARYING H-ION CONCENTRATION

CULTURE NO.	INITIAL P_H	NOTES
1......	2.81	No growth; slight brownish precipitate
2......	3.07	No growth; slight brownish precipitate, more than in no. 1
3......	3.20	No growth; slight brownish precipitation, more than in no. 2
4......	3.47	Growth (?); agglutinated precipitate
5......	4.14	Growth, but slight and not very green
6......	4.62	Good growth; distinctly green but with a yellowish cast
7......	4.94	Good growth; more than in no. 6; healthier and more vigorous; slightly yellowish
8......	5.05	Good growth; more than in no. 7; not as bright green as in no. 9
9......	5.73	Probably the best growth; one culture contaminated with a fungus; bright green
10......	6.31	Growth less than in no. 9; about like no. 8
11......	6.68	No growth
12......	6.95	No growth
13......	7.10	No growth
14......	7.13	No growth

TABLE III

EFFECT OF VARYING H-ION CONCENTRATION; EXPERIMENT 1

No.	P_H AT START	P_H AT END			DRY WEIGHT OF CROP (MG.)			AVERAGE CROP (MG.)
		A	B	C	A	B	C	
1.....	2.81	2.84	2.84	....	−0.9	−0.9	....	0.0
2.....	3.07	3.04	3.04	....	−0.2	+0.4	....	0.1
3.....	3.20	3.11	3.12	3.14	+0.8	+0.8	−0.8	0.4
4.....	3.47	3.41	3.41	3.41	−0.4	−0.5	−1.2	0.0
5.....	4.14	4.61	4.48	4.35	19.9	13.3	9.0	14.1
6.....	4.62	4.83	4.77	4.83	65.4	52.6	37.3	51.8
7.....	4.94	5.05	5.04	4.99	93.4	84.4	62.4	80.1
8.....	5.05	5.10	5.10	5.10	105.8	116.9	67.8	96.8
9.....	5.73	6.69*	5.78	5.90*	177.9*	190.7	175.7*	190.7
10.....	6.31	6.30	6.37	6.34	146.1	110.6	149.5	135.4
11.....	6.68	6.66	6.50	....	006.2	005.9	....	006.0

* Contaminated.

an initial H-ion concentration in the vicinity of P_H 5.0, while in the very acid and somewhat alkaline cultures no growth was detected. The details of these observations are recorded in table II.

On April 29, fourteen days after inoculation, determinations were made of the dry weight of the crop produced in each culture, and of the final H-ion concentration of each solution. Each individual culture was added to a large Pyrex test-tube and the algal cells thrown down in the centrifuge. A portion of the clear supernatant liquid was poured off for P_H determination, and the residue made slightly acid in order to dissolve any precipitated salts. The algal cells were then

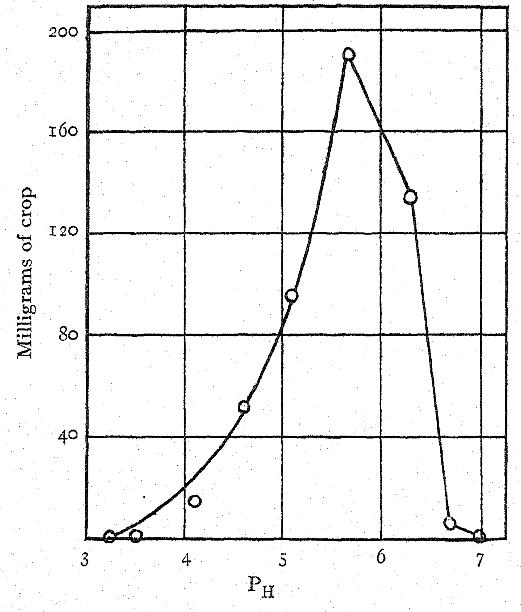

FIG. 2.—Growth-P_H curve of Experiment 1 with normal culture solution

thrown on a weighed Gooch crucible provided with a thoroughly washed asbestos mat, and the weight of the crop determined after drying *in vacuo* at 80° C. for 18 hours. The results are given in table III. In cases where contaminations occurred, the dry weights are not included in the averages. The crops were not determined in culture solutions nos. 12, 13, and 14, as no growth was apparent in any of the flasks.

It should be noted that the H-ion concentrations of the solutions remained very constant, even when there was considerable growth of the alga. In no case, except where contamination was a factor,

did the final P_H determination vary from the initial by more than 0.5, and in only seven cases was the variation greater than 0.1.

The data for the crop determinations are plotted in fig. 2, and show, so far as this experiment is concerned, that maximum growth occurred at P_H 5.7. The acid limit may be placed at about 3.4, and the alkaline at about 6.7. The curve representing the growth from 3.4 to 5.7 is very uniform.

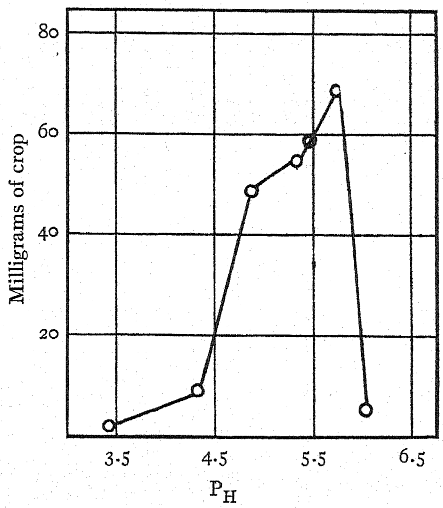

FIG. 3.—Growth-P_H curve of Experiment 2 with normal culture solution.

Experiment 2

In the experiment just described, the H-ion concentration values in the region of maximum crop production were rather widely separated, as an examination of the composition-P_H curve, fig. 1, will readily show. In an effort to obtain data on the growth of *Chlorella* over a closer P_H range in this region, and to check the results already secured, the experiment was repeated, using the same solutions and methods of procedure, but introducing a few more points in the P_H range. Culture solutions nos. 1, 2, and 3 on the acid end of the series, and nos. 13 and 14 on the alkaline end were omitted in this test because of the entire absence of growth in them. Four replicates of each culture solution were prepared as before, one flask of each lot being used for the initial H-ion concentration determination, and the remaining three being inoculated. The P_H determinations were made by means of the colorimetric method and checked in a few instances with electrometric determinations.

The inoculations were made on October 29, using 0.5 cc. of a suspension of *Chlorella* cells for each flask. Growth occurred in this series over practically the same range of P_H as in the previous ex-

periment, the best growth being at 5.7. At the end of three weeks the usual crop determinations were made. H-ion concentration determinations at this time showed very little change in the solutions. The data for the second experiment are presented in table IV, and are also shown graphically in fig. 3. The results confirm the point of maximum growth of *Chlorella* in this nutrient solution at P_H 5.7.

TABLE IV

DATA FOR EXPERIMENT 2: EFFECT OF P_H ON GROWTH

Solution no.	Composition: 25cc. solution *A* plus			Initial P_H	Final P_H			Average crop (mg.)
	M/7.5 H_3PO_4 (cc.)	M/7.5 KH_2PO_4 (cc.)	M/7.5 K_2HPO_4 (cc.)		A	B	C	
4	1	24.000	....	3.4	3.2	3.3	3.2	01.5
5	....	25.000	....	4.2	4.3	4.3	4.1	08.8
6	....	24.375	0.625	4.9	5.3	5.3	5.2	48.1
7	....	23.750	1.250	5.3	5.3	5.4	5.3	54.4
8	....	22.500	2.500	5.5	5.5	5.5	5.5	59.3
8a	....	21.250	3.750	5.7	5.7	5.7	5.9	68.5
9	....	17.500	7.5	6.0	6.2	6.2	6.2	05.6
9a	....	13.750	11.25	6.3	....	....	....	....
10	....	10.000	15.0	6.6	....	....	....	....
10a	....	7.500	17.5	6.7	....	....	....	....
11	....	5.000	20.0	6.8	....	....	....	....
12	....	1.250	23.75	7.2	....	....	....	....

PRECIPITATION OF SALTS OF SOLUTIONS

In both of the experiments, all the solutions on the acid side of P_H 5.7 were perfectly clear, whereas considerable precipitation occurred on the other side of this point. The precipitate consisted chiefly of magnesium and calcium phosphates, and it seems probable that the iron, supplied as ferric sulphate, was also largely thrown down. In the second experiment the amount of precipitation was greater than in the solutions of the first experiment, due no doubt to the lower temperature obtaining in the fall of the year. This fact probably accounts for the sharp drop in fig. 3, from 5.7 to 6.0. Because of this marked precipitation, the question arose as to whether lack of growth in these solutions was purely an effect of P_H, or might be due to the unavailability of some essential mineral elements. If the H-ion concentration alone were the factor involved, then

Chlorella should grow in these solutions containing heavy precipitates, provided the reaction was adjusted to the vicinity of P_H 5.5. On the other hand, magnesium, calcium, or iron may have been completely precipitated, resulting in solutions unfavorable for growth. Qualitative tests showed a mere trace of each in the filtered solutions. From the unpublished results of SCHRAMM,[2] it does not seem likely that lack of calcium would be a factor, as he has shown that good growth can be maintained in solutions from which calcium has been entirely omitted. Iron and magnesium, however, cannot be so completely eliminated without producing an effect. The following experiment was therefore devised to throw some light on this question.

Experiment 3: Growth in readjusted solutions

A liter of the complete nutrient solution was prepared in the usual manner, using a phosphate buffer mixture which gave an initial H-ion concentration of P_H 6.8. A copious precipitate formed, and the solution was allowed to stand until this had settled out completely. The precipitate was then filtered off and the clear filtrate adjusted to P_H 5.5, with concentrated HCl of low iron content (0.0001 per cent), 2.5 cc. of the acid being required. Fifty cc. of adjusted solution was introduced into each of sixteen flasks. These were divided into four lots of four flasks each, and treated as follows: (1) no additions; (2) one drop of $Fe_2(SO_4)_3$ solution to each flask; (3) 2 cc. 0.4 per cent $MgSO_4$ solution added to each flask; (4) 2 cc. 0.2 per cent $CaCl_2$ added to each flask. The flasks were then sterilized and inoculated with a suspension of *Chlorella* cells. The P_H after sterilization was 5.3.

As a check for this series, a number of flasks containing the unadjusted solution of P_H 6.8 were prepared in the usual manner. Some of these were inoculated at once; the contents of others were filtered through sterile filters into sterile flasks and then inoculated. After twelve days' growth, observations were recorded as follows: (1) fair growth, cells green at first but turning brown; (2) very good growth, bright green, healthy appearance; (3) good growth, bright green at first, turning to yellow-green; (4) slight growth, green at first, becoming practically colorless; (checks) no growth in unad-

[2] Paper presented before the Botanical Society of America, Boston Meeting, 1922.

justed solutions with heavy precipitate present; slight trace in unadjusted solution, filtered sterile. At this time P_H determinations showed that practically no change had occurred in the reaction of the solutions. Crop determinations were made, with the results shown in table V.

It is apparent that the unadjusted solution at 6.8 will not support growth. The small amount of growth in the filtered check may have been due to iron dissolved from the filter paper. The same solution, however, with reaction adjusted to P_H 5.3 permits of considerable development of *Chlorella*. In the light of later experiments

TABLE V

GROWTH IN READJUSTED SOLUTIONS

Solution	Treatment	Initial P_H	Final P_H	Average crop (mg.)
Unadjusted solution........	None	6.8	....	None
Unadjusted solution........	Filtered	6.7	....	01.8
Filtered and adjusted (1)...	None	5.3	5.1	05.9
Filtered and adjusted (2)...	+Fe	5.3	5.3	14.5
Filtered and adjusted (3)...	+Mg	5.3	5.3	13.0
Filtered and adjusted (4)...	+Ca	5.3	5.1	04.8

reported in this paper, it seems probable that this increase in growth is not due merely to the change in reaction, but to slight traces of iron added in the acid used in adjusting the solution after filtering. The addition of a small amount of iron or magnesium to the adjusted solution increased growth two or threefold, while the addition of calcium apparently depressed the growth. In solutions of P_H 6.0 and above, therefore, the precipitation of iron is a factor in the growth of the organism. Magnesium also may be involved. A trace of iron may have contaminated the magnesium solution used in adding this element, although it was not apparent from a qualitative test. Lack of calcium apparently is not a factor in prohibiting growth, while the depressing effect of its presence may be due to toxicity.

Experiment 4: Growth in Beijerinck's nutrient solution

One of the mineral nutrient solutions frequently used for the culture of green algae is that of Beijerinck, the composition of which, as modified by Moore, is as follows:

NH_4NO_3	0.5 gm.
K_2HPO_4	0.2 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
$CaCl_2$	0.1 gm.
$Fe_2(SO_4)_3$	Trace
Distilled H_2O	1000 cc.

This solution, either with or without the addition of agar or glucose, has proved very satisfactory for pure culture work with these forms. The reaction of the solution, however, is practically neutral, which would seem, from the results obtained above, to be unfavorable for growth. It seemed desirable, therefore, to examine the growth of *Chlorella* in this solution at various H-ion concentrations. The following experiment was thus devised.

TABLE VI

GROWTH IN ADJUSTED BEIJERINCK'S SOLUTION

SOLUTION	INITIAL P_H	FINAL P_H	AVERAGE CROP (MG.)
No sugar	6.9	6.4	2.9
	6.6	6.4	2.2
	6.5	6.2	2.7
	6.3	5.8	2.5
	5.3	4.0	2.1
1 per cent glucose	6.2	Less than 3.1	157.3
	5.8	Less than 3.1	137.0
	5.1	Less than 3.1	118.6
	4.1	Less than 3.1	116.2

A quantity of Beijerinck's solution was prepared, to half of which 1 per cent glucose was added. The P_H of the two portions, with and without glucose, was 6.9 and 7.0 respectively. The two solutions were then introduced individually into a number of Erlenmeyer flasks, in quantities of 50 cc. in every case, and the flasks sterilized. The solutions without glucose showed practically no change in P_H after sterilization; the solution with glucose had a P_H of 6.2 after sterilization. Several lots of the two solutions were adjusted in varying degrees toward the acid end of the P_H range by the addition of sterile HCl, and the whole series then inoculated with a suspension of *Chlorella* cells. At the end of a month determinations were made of the H-ion concentration of the solutions and of the dry weight of crops produced. The data are shown in table VI.

It is apparent that, although Beijerinck's solution may be nearly neutral when inoculated, it develops an acid reaction as growth proceeds, due probably to an unequal absorption of ions from the solution. Nitrogen is here supplied as NH_4NO_3, and it is conceivable that the NH_4 ion may be absorbed in excess of the NO_3 ion, thus producing the change in P_H. It should also be noted that there is not an adequate buffer system in this solution to maintain the reaction, as was provided in the experiments reported earlier in this paper. The fact that Beijerinck's solution will maintain a good vigorous development of green algae, therefore, is not evidence that a P_H of 6.8 or 7.0 is favorable for the growth of these forms.

Solubility of ferric iron in buffered culture solutions

With the idea in mind that the results obtained in previous experiments may have been caused by the insolubility of iron at the higher P_H values, some experiments were performed to determine the extent of this precipitation. At the same time an attempt was made to keep the iron in solution by means of salts of certain organic acids. The fact that lack of iron is a factor in alkaline solutions is indicated in Experiment 3, where the addition of iron to an alkaline culture solution after filtering and adjusting to a P_H of 5.5 increased the growth markedly over the check to which no iron had been added.

Phosphate solutions identical with those used in the previous experiments were mixed so as to give, when diluted with an equal amount of water, a buffer solution having a P_H of 7.0. At the same time 1 gm. of ferric sulphate was dissolved in 100 cc. of water, to which had been added three drops of concentrated hydrochloric acid. Various mixtures were then made, as follows:

Buffer solution (cc.)	Iron solution (cc.)	Water (cc.)	Other additions
25...........	1	25	None
25...........	1	25	0.5 gm. Na citrate
25...........	1	25	0.5 gm. K tartrate
25...........	1	25	0.5 gm. NaK tartrate

The last three mixtures were prepared in duplicate. At the time of mixing, all the solutions were turbid except the ones to which sodium citrate had been added. The next morning all had a small amount of flocculent precipitate with a clear supernatant liquid ex-

cept the ones with sodium citrate. In these no precipitate could be detected. These latter solutions were distinctly yellowish in color.

At this time the solutions were filtered through filter papers which had been washed thoroughly with distilled water, and the filtrates tested for iron by the procedure of MARRIOTT and WOLF (5).

The writers would like to point out that the extreme sensitiveness of this test for ferric iron makes it of great value in a study of the physiological effect of iron on plant growth, as amounts of iron well below that necessary for growth may accurately be determined. MARRIOTT and WOLF found that as little as 0.0005 mg. of ferric iron could readily be detected, and that differences such as between 0.002 and 0.003 mg. are easily determined. In our own experiments we also approached this same degree of accuracy. For instance, 0.00025 mg. of Fe could easily be distinguished from the blank and also from 0.0005 mg. It is imperative of course that the reagents used be free from iron, or at least have such a low iron content that a blank prepared from them have the very faintest trace of color when compared with a tube of water. The method slightly modified by us is as follows.

One cc. of concentrated hydrochloric acid of low iron content (0.0001 per cent) was placed in a 50 cc. graduated cylinder, and 10 cc. of a 10 per cent solution of KSCN, and 10 cc. or less of the solution to be tested added. The volume was then made up to 25 cc. with distilled water, and then to 50 cc. with acetone, and mixed. Comparison was made directly in this cylinder with a series of standards prepared in a similar manner in other 50 cc. graduated cylinders, and containing known amounts of iron. It was found convenient to place the cylinders on a glass plate supported above white paper, and compare the colors by looking down through the solutions. The concentration of hydrochloric acid used prevents the interference of phosphates (5). The standards were prepared from a stock solution made in the usual manner by dissolving pure iron wire.

The results of the preliminary tests on the filtered buffer solutions are shown in table VII. In the case of the check, the precipitate on the filter was dissolved in a little HCl and diluted to 50 cc., and also tested for iron. From this table it is seen that, after standing

over night, only about 1/100 of the iron is left in the phosphate buffer solution to which no organic salt was added. Practically all the iron is in the precipitate which forms. In the solution to which sodium citrate was added all of the iron remained in solution. The two tartrate salts used do not appear to be effective in holding the iron in solution.

TABLE VII

SOLUBILITY OF IRON IN PHOSPHATE BUFFER SOLUTIONS AT P_H 7.0

Solution	Fe in 50 cc. (mg.)	P_H	
		Brom cresol purple	Phenol red
Check (filtrate)	0.028	6.9	6.9
Check (precipitate dissolved in HCl and diluted to 50 cc.)	2.800	....	....
Solution +0.5 gm. Na citrate (filtrate)	2.800	7.0	7.1
Solution +0.5 gm. K tartrate (filtrate)	0.056	6.9	6.9
Solution +0.5 gm. NaK tartrate (filtrate)	0.028	6.9	6.9

TABLE VIII

EFFECT OF SODIUM CITRATE ON SOLUBILITY OF IRON; OBSERVATIONS AFTER ABOUT 20 HOURS

Solution	Appearance	Fe in 50 cc. (mg.)	P_H
Check	White flocculent precipitate	Less than 0.028	7.4
+0.2 gm. citrate	Clear	....	....
+0.2 gm. citrate +Mg	Clear	....	....
+0.1 gm. citrate	Clear	....	....
+0.1 gm. citrate +Mg	Clear	....	....
+0.01 gm. citrate	Clear	....	....
+0.01 gm. citrate +Mg	Clear	....	....
+0.005 gm. citrate	Clear	2.8	7.4
+0.005 gm. citrate +Mg	Very slight turbidity	2.8	7.4

In another test using varying amounts of sodium citrate at a P_H of 7.4, it was found that this salt in as low a concentration as 0.005 gm. per 50 cc. of solution would hold the 2.8 mg. of iron in solution also. In this experiment magnesium sulphate was added in the same concentration as used in the culture solutions, to see whether this affected the solubility of iron. The results are given in table VIII. It is evident that only a small amount of the sodium citrate is necessary to keep the iron in solution, and also that magnesium does not interfere with its action.

Experiment 5: Effect of sodium citrate on availability of iron in normal culture solution

On the basis of the preceding experiments, another series of cultures was set up, using the same procedures as followed in Experiments 1 and 2, except that sodium citrate was added. The concentration of sodium citrate used was 0.4 gm. per liter of solution *A*. The final concentration was thus 0.2 gm. per liter of the culture solution as finally prepared.

In place of ferric sulphate, a standard solution of ferric chloride, prepared from pure iron wire and containing 0.1 mg. Fe per cc. was used. This was added at the rate of 8 cc. per liter of solution *A*, which amounts to 0.02 mg. Fe per culture flask containing 50 cc. of the final culture solution. As the actual tests of the solutions in Experiments 5 and 6 showed 0.0575 and 0.050 mg. Fe respectively per culture, it was thought that some iron must have been present as an impurity in the other reagents. From subsequent tests made on culture solutions with and without glucose, it appears that most of the additional iron was derived from impurities present in this sugar.

Six replications of the culture solutions were prepared for each P_H. Five of these sets were inoculated with *Chlorella* cells, and the remaining set used for initial H-ion determinations and iron tests as shown in table IX. On making the iron tests, it was found that, in spite of the presence of sodium citrate, the iron had unfortunately disappeared out of the more alkaline solutions. After standing over night no iron was found in the filtrates from cultures 8–14, and only small amounts in nos. 6 and 7. The others showed strong tests which indicated that none of the iron had been lost. The loss of iron in the more alkaline solutions may be accounted for by the fact that in these solutions an amorphous precipitate of calcium phosphate forms, and on this precipitate the iron is adsorbed. Thus, although the iron is present in a soluble form, it is removed from solution. This phenomenon should be distinguished from that occurring in the check solutions in the experiments just described (tables VII and VIII), where chemical precipitation of the iron occurs at the higher P_H values.

Providing that the iron removed by adsorption in this manner is unavailable for the growth of *Chlorella*, it was to be expected that

this series of culture solutions would give results little different from those obtained in Experiments 1 and 2; such proved to be the case. In making the inoculations of cultures 6–14 in each series of this experiment, an attempt was made to overcome this difficulty by adding in the cell suspension an amount of iron equivalent to that originally present. While, as the final iron tests show, most of this iron was also removed from solution, it appears that the shape of the growth curve is affected. The decline in the curve on the alkaline

TABLE IX

EFFECT OF SODIUM CITRATE ON AVAILABILITY OF IRON IN NORMAL CULTURE SOLUTION

SERIES NO.	P_H AT START	P_H AT END					FE INITIAL	FE FINAL MG. PER CULTURE SOLUTION			AVERAGE CROP (MG.)
		A	B	C	D	E		A	B	C	
1...	3.60	.....*	3.7	3.7	3.70	3.7	+	........*	0.0575	....	1.95 ±0.23†
2...	4.10	5.30	5.2	5.2	5.30	5.3	......	0.0175	....	....	46.54 ±0.62
3...	4.70	5.40	5.4	5.4	5.5	5.5	......	0.0175	....	....	72.96 ±1.55
4...	4.90	5.55	5.55	5.6	5.55	5.6	......	0.0125	....	....	87.38 ±1.24
5...	5.4	5.40	5.4	5.4	5.4	5.4	......	0.00675	....	....	84.14 ±1.30
6...	5.6	5.50	5.5	5.5	5.5	5.5	+	0.0075	0.015	....	95.40 ±1.60
7...	5.9	5.75	5.75	5.75	5.75	5.75	+	0.0175	0.020	....	90.86 ±1.30
8...	6.1	6.20	6.2	6.2	.....*	6.2	.....*	0.00177	<0.00125	....	83.52 ±2.59
9...	6.4	6.4	6.4	6.4	.....*	6.4	.....*	<0.00125	<0.00125	....	43.85 ±1.10
10...	6.7	....*	6.7	6.6	6.7	6.6	......	........*	<0.00125	<0.00125	7.07 ±1.19
11...	6.9	6.9	6.9	.....*	6.9	6.9	......	<0.00125	<0.00125	....	1.07 ±0.33
12...	7.05	7.0	.....*	7.1	7.1	.....*	......	<0.00125	....	....	0.83 ±0.27
13...	7.30	7.15	7.1	7.15	....*	......	......	0.0075	....	0.0075	1.9 ±0.83
14...	7.40	7.2	7.2	7.25	7.15	......	.....*	0.0125	<0.00125	....	3.1 ±1.16

* Culture contaminated.

† Probable error of the mean calculated by means of Bessel's formula.

side is not so steep as in Experiments 1 and 2. The data are given in table IX and shown graphically in fig. 4. In this experiment, as well as in the following one, the cultures, after inoculation, were placed in the greenhouse but protected from direct sunlight. The cultures of each series were so distributed as to overcome effects due to unequal lighting. Crop determinations were made two weeks after inoculating.

The greatest growth is at P_H 5.7 as before, and falls off very rapidly as the solutions become more alkaline. As in the case of the previous experiments, however, this cannot be looked upon as the true effects of H-ion concentration on growth because of the variation in the iron content of the nutrient solution. This is brought out

very strikingly by the data obtained in Experiment 6, in which we were successful in keeping the iron in solution in all cultures.

Experiment 6: Effect of sodium citrate on availability of iron in culture solution lacking calcium

As the work of SCHRAMM, previously referred to, shows that very little if any calcium is necessary for the growth of *Chlorella*, it was decided to set up a series in which the calcium nitrate of the nutrient

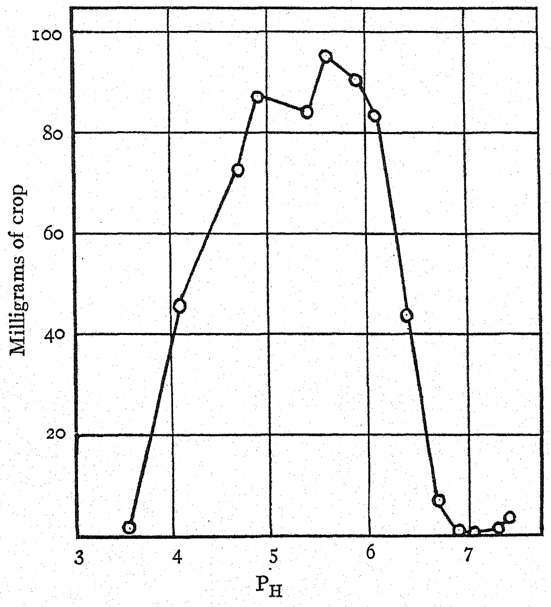

FIG. 4.—Growth-P_H curve of Experiment 5 with normal culture solution to which sodium citrate was added.

solution was replaced by ammonium nitrate containing an amount of nitrogen equivalent to that supplied by the calcium nitrate in the other series. It was found that in solutions prepared in this manner no precipitate forms at any of the H-ion concentrations used. Under these conditions, with sodium citrate present, the iron is neither precipitated chemically nor removed by adsorption. The growth should therefore be a function of the H-ion concentration.

Six replications of the culture solutions were prepared, and except

for the substitution mentioned, the same procedure was followed as in Experiment 5. In preparing the suspension of algal cells, instead of a balanced solution, a 0.6 per cent NaCl solution was used. Crop determinations were made two weeks after inoculating. The data for this experiment are summarized in table X. It will be noted that the initial iron tests show as much iron in the most alkaline solution

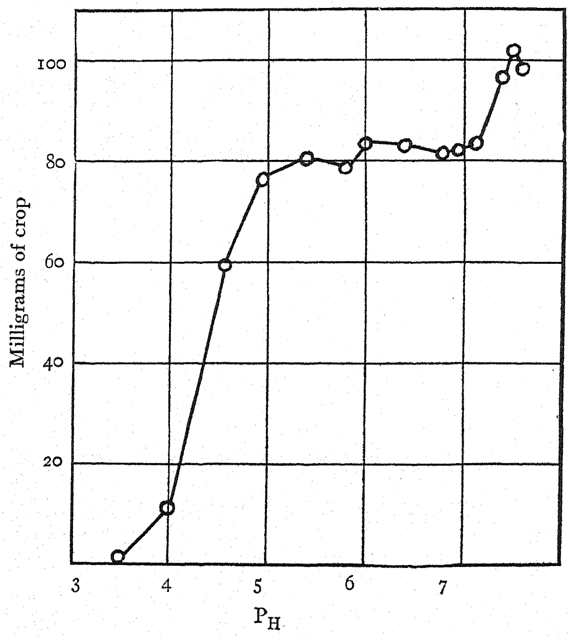

FIG. 5.—Growth-P_H curve of Experiment 6 in which calcium of normal culture solution was replaced by ammonium, and sodium citrate was added to keep the iron in solution.

(7.5) as in the most acid solution (3.5). The most interesting thing shown by these data, however, is the effect of the H-ion concentration on growth. Instead of a rapid falling off in the growth from P_H 5.7, it continues at approximately the same rate as the more alkaline solutions are reached, and even increases still more at the values of 7.4 and 7.5 (fig. 5).

The data for this experiment show that in most cases there is a slight increase in the acidity of the solution, probably due, as

postulated earlier, to a differential absorption of the ions of ammonium nitrate. In order to test this experimentally, analyses of the solutions were made at the end of the experiment for ammoniacal nitrogen and total nitrogen. The difference between these two results will give the amount of nitrate nitrogen. Ammoniacal nitrogen was determined by means of a modified Folin method, and the total nitrogen with Devarda's alloy.

TABLE X

EFFECT OF SODIUM CITRATE ON AVAILABILITY OF IRON IN CULTURES MINUS CALCIUM

SERIES NO.	P_H AT START	P_H AT END					FE INITIAL MG. PER CULTURE	FE FINAL MG. PER CULTURE *A*	AVERAGE CROP (MG.)
		A	B	C	D	E			
1	3.50	3.6	3.6	3.7	3.7	3.6	0.050	0.044	1.08±0.09
2	4.00	4.2	4.2	4.2	4.2	4.2	....	0.048	11.06±1.68
3	4.65	4.4	4.4	4.45	4.3	4.4	....	0.044	59.30±1.23
4	4.95	4.6	4.6	4.45	4.4	4.6	....	0.044	76.52±1.15
5	5.4	4.8	4.7	4.7	4.65	4.7	....	0.044	80.88±2.17
6	5.8	5.4	5.4	5.4	5.4	5.4	....	0.0180	78.84±1.01
7	6.0	5.65	5.65	5.65	5.65	5.75	....	0.0230	83.32±0.80
8	6.4	6.0	6.1	6.0	6.2	6.1	....	0.0230	83.02±1.31
9	6.8	6.4	6.4	6.4	6.4	6.4	....	0.0345	81.48±0.95
10	6.95	6.6	6.6	6.5	6.5	6.6	....	0.0184	82.20±2.00
11	7.15	6.8	6.8	...*	6.8	6.8	....	0.0253	83.40±3.18
12	7.4	6.9	6.9	6.9	...*	6.9	....	0.0184	96.45±3.67
13	7.5	6.9	7.0	7.0	7.0	6.95	0.050	0.0253	101.96±4.32
14	7.6	7.0	7.0	7.0	7.0	7.0	0.050	0.0184	98.24±2.55

* Culture contaminated.

From table XI it appears that in all cases except nos. 1 and 2, in which cultures the reaction did not become more acid, there is a greater absorption of the ammonium ion than the nitrate ion. This would account for the reaction becoming more acid, and would also appear to justify our explanation of the increased acidity in the case of Beijerinck's solution. The varying amounts absorbed are due to differences in the amount of growth, and perhaps also to the differences in H-ion concentration. It is recognized, of course, that in alkaline solutions the liberation of gaseous ammonia may introduce an error in the determinations. As our most alkaline cultures were only slightly more alkaline than neutrality, however, it does not seem likely that this would occur.

In order to determine whether the lack of calcium in the culture solution caused any decrease in the crop, two series of cultures were prepared at the same P_H. One series contained calcium, and the composition was the same as in culture 5, Experiment 5; the other series

TABLE XI

DIFFERENTIAL ABSORPTION OF IONS OF AMMONIUM NITRATE FROM CULTURE SOLUTIONS

Culture no.	Ammoniacal nitrogen (mg.)		Nitrate nitrogen (mg.)	
	In solution	Absorbed	In solution	Absorbed
1 D	4.10	0.15	3.89	0.36
2 D	3.83	0.42	3.75	0.50
3 D	2.58	1.67	3.15	1.10
4 D	2.09	2.16	3.14	1.11
5 D	1.77	2.48	2.32	1.93
6 D	1.37	2.88	1.48	2.77
7 D	1.42	2.83	3.89	0.36
8 D	2.04	2.21	3.74	0.51
9 D	2.24	2.01	3.09	1.16
10 D	2.40	1.85	3.81	0.44
11 D	2.19	2.06	4.11	0.14
12 D	1.91	2.34	4.37	....
13 D	1.78	2.47	4.38	....
14 D	1.69	2.56	4.44	....
Original solution	4.25	....	4.25*	....

*Calculated.

TABLE XII

EFFECT OF OMITTING CALCIUM FROM CULTURE SOLUTION AND REPLACING IT BY AMMONIUM

Culture no.	Calcium present		No calcium	
	Crop (mg.)	P_H (final)	Crop (mg.)	P_H (final)
1	220.8	5.6	257.0	5.1
2	228.5	5.6	243.8	5.0
3	242.8	5.6	273.8	4.8
4	224.5	5.6	265.9	4.7
Average	229.1 ±3.2	5.6	260.1 ±4.3	4.9

contained no calcium, and the composition was the same as culture 5, Experiment 6. The initial P_H was 5.4 in both series, and no precipitate was present in any of the solutions. The cultures were inoculated March 17, 1925, and the crops and final P_H determinations

were made April 23 following. The results presented in table XII, instead of showing a decrease when calcium is omitted, show a marked increase over the series in which calcium is present. It is of interest to note that, while in the series in which calcium is present there is a slight increase in P_H, in the other series where calcium is replaced by ammonium there is a marked decrease. This is in keeping with the results obtained in Beijerinck's solution and in Experiments 5 and 6. This increase in acidity in cultures containing ammonium nitrate has already been discussed. The greater increase in the Beijerinck's solution is due to the fact that this is not so well buffered as in the other experiments.

Discussion

From a consideration of the experiments reported in this paper, it is obvious that if precautions are not taken to keep the iron of the culture medium in solution, the results obtained by varying the H-ion concentration will not represent the true effect of this factor on growth. Within a certain acid range, of course, no special efforts are needed to retain the iron in a soluble form; it is in the more alkaline solutions that it may be precipitated and its concentration so reduced as to be less than that required for growth. In Experiments 1 and 2, for instance, where the iron was precipitated chemically and probably also adsorbed to some extent, and in Experiment 5 where it was no doubt removed mostly by adsorption in the more alkaline cultures, there appeared to be a definite maximum for growth at about P_H 5.7, from which point the growth rapidly declined as the reaction became more alkaline, until at 7.0 there was no growth. In view of the results obtained in Experiment 6, however, where the iron was kept in solution at all P_H values, and also from the fact that most of the iron was found to be lost from the alkaline cultures of Experiments 1, 2, and 5, it is seen that except for reactions more acid than P_H 5.7, this does not represent a real effect of H-ion concentration on growth. In Experiment 6 growth continues to increase, even at 7.5. The alkaline limit for the growth of *Chlorella* has not been determined, therefore, and it remains for further work to decide this point.

The unavailability of iron in nutrient solutions at the higher P_H

values has been discussed by a number of workers, among whom might be mentioned TOTTINGHAM and RANKIN (8), and REED and HAAS (7). Earlier literature will be found cited in these papers. The general facts brought out are that iron is precipitated at the lower H-ion concentrations; that its precipitation is more rapid the lower the H-ion concentration; that the iron of certain iron salts is less likely to be precipitated than that of others; and that certain salts of organic acids tend to keep the iron in solution. Among the most favorable forms of iron appears to be ferric citrate, and it has been shown that this salt tends to remain in solution. DUGGAR (3) has shown that "soluble ferric phosphate" is an excellent source of iron, but as this substance is prepared by mixing ferric citrate and sodium phosphate (2), it is probably equivalent to adding ferric citrate to solutions in which the other ions are already present. In our experiments, where sodium citrate was added to the culture solutions, ferric citrate was probably present. It is possible that, if the iron in this case is present in colloidal solution in a condition of high dispersity, as suggested by DUGGAR, the citrate ion may act as a peptizing agent tending to maintain this condition. An excess of sodium citrate as used in our experiment may therefore be desirable. REED and HAAS show that where sodium citrate is added to a culture solution the iron is retained in solution, so that an "abundant" test is obtained with KSCN at P_H 7.6, while the control shows "none." While this result was qualitative, it shows that considerable iron at least was held in solution. Their test seems to differ from ours, in that they report a white turbidity where the sodium citrate was added, while ours was clear with a faint yellowish color.

An important point brought out in Experiment 5 is the effect on the iron concentration of an amorphous precipitate, calcium phosphate, even when the iron is present in a so-called soluble form. As this precipitate is frequently formed when nutrient solutions are made alkaline, it may account for the discordant results reported in the literature as to the availability of certain forms of iron.

As regards the solubility of iron in alkaline cultures where no sodium citrate was added, the high concentration of phosphates used to buffer the solutions may have decreased the solubility of iron, if this were present in the solutions as ferric phosphate, because of

their effect on the solubility product. We might therefore expect less iron in these solutions than in ordinary unbuffered culture solutions at the same P_H. The high concentration of phosphate does not appear to affect the solubility of iron when sodium citrate is present.

In investigating the availability of iron at varying concentrations of the H-ion, changes in the P_H value of the solution during the course of the experiment should be taken into account. This is especially true in unbuffered solutions. This is brought out by REED and HAAS in connection with the use of ammonium salts of organic acids which caused a marked increase in the H-ion concentration. Experiment 4, in which Beijerinck's solution was used, showed a marked increase in H-ion concentration during the growth of the organism, due, as subsequent tests showed, to the rapid absorption of the ammonium ion in preference to the nitrate ion of the ammonium nitrate. This increase in acidity will cause a marked increase in the solubility of iron. In strongly buffered solutions, such as used in Experiment 6, this effect is slight.

Summary

1. The rate of growth of *Chlorella* in highly buffered nutrient solutions is directly influenced by the H-ion concentration when the P_H is less than 5.7. The acid limit for growth of this organism in the culture media tested is 3.4.

2. In solutions in which the P_H is higher than 5.7, the availability of iron becomes a limiting factor for growth.

3. Certain organic compounds, especially sodium citrate, are effective in holding iron in solution in alkaline buffer mixtures of 7.4. When calcium is present, however, the iron is completely removed from such solutions by the precipitated calcium phosphate, probably as the result of adsorption.

4. Since calcium is not essential for the growth of *Chlorella* it can be omitted entirely from the culture solution. Iron can then be maintained in alkaline solutions in a form available for growth by the addition of sodium citrate. In such solutions maximum growth occurred at 7.5. The alkaline limit for growth has not as yet been established.

5. In unbuffered nutrient solutions, marked changes in the H-ion concentration may be brought about by the unequal absorption of ions, resulting in increased acidity, which in turn renders the iron available for growth.

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SPOROGENESIS IN REBOULIA HEMISPHAERICA

MARY CONSTANCE BLAIR

(WITH PLATES XXX, XXXI AND THREE FIGURES)

Historical review

The earliest contributions upon sporogenesis, by HOFMEISTER, LEITGEB, and LECLERC DU SABLON, were upon morphological characters wholly. FARMER'S (9) paper in 1893 marked the beginning of cytological investigation in the Bryophytes. *Aneura pinguis* and *Pallavicinia decipiens* were shown to reduce by a quadripolar spindle, which effected a simultaneous distribution of the chromatin to four separate points. In 1894 FARMER (10) published a substantiating investigation for *Pallavicinia*, but in *Aneura* he found that the quadripolar spindle was transient, and that it gave way to two simultaneous spindles that effected reduction by joint action.

FARMER'S (11) greatest contribution appeared in 1895, as a comparative study of several types, with confirmatory observations upon additional species. *Fossombronia Dumortieri* and *Pellia epiphylla* (anacrogynous Jungermanniales) were found to possess a prophasic quadripolar structure, which in metaphase did not become a quadripolar spindle. The evolved bipolar spindle arose by the fusion, in pairs, of the arms of this quadripolar structure; this was effected by the activity of four marked centrospheres or centrosomes with astral radiations. A complete septum followed this spindle. With no period of interkinetic rest, two simultaneous bipolar spindles completed reduction.

In the Marchantiales, *Fegatella conica* showed no lobing of the mother cell and no quadripolar figure. The early heterotypic spindle was triangular, having the block of chromosomes at one angle, and a very distinct centrosphere (with aster) at each of the other angles. By their progressive migration, the bipolar spindle was established with a straight axis. With no period of interkinetic rest, duplicate simultaneous spindles completed the reduction division. Cell plates preceded spore wall formation. Confirmatory examples were af-

forded in *Riella*, *Plagiochasma*, and *Marchantia*. The results secured in this investigation led FARMER to postulate a theory of phylogenetic advance in the reduction processes of plants. To him the Bryophytes furnished several transitional phases of marked significance.

MOORE (25) investigated *Pallavicinia Lyellii*, with very different results from those attained by FARMER in *P. decipiens*. The first spindle was bipolar, and had no cell plate. Eight tetrads arose from a split spireme. The kinoplasm arose as caps attended by marked nuclear distortion, and the nuclear membrane was resolved into spindle fibers. There was no interkinetic rest. No nuclear membrane appeared. The subsequent, simultaneous homotypic spindles established cell plates.

DAVIS (7) investigated *Anthoceros laevis* L. and discovered no centrospheres and no quadripolar spindle, but there was a true bipolar spindle. The daughter nuclei attained a fully developed resting condition. Homotypic spindles followed. The rather unusual findings included: (1) a spireme unchanged in character post-synaptically; (2) the laying down of the spore wall by a Hautschicht layer without the aid of spindle fibers or the formation of cell plates.

DAVIS (8) made a detailed investigation of *Pellia epiphylla*. He found the quadripolar spindle to be a transitory feature of prophase, and to be unattended by centrospheres. Granular protoplasm was the only form of kinoplasm permanent in the cell. Double longitudinal splitting of the chromatin was not found, and meiosis was effected by one heterotypic followed by two homotypic spindles. In other stages of ontogeny, centrospheres and polar caps were found.

BEER (2), working on *Riccia glauca*, found a continuous postsynaptic spireme, which was thickened at the expense of a nucleolus that was always distinct among indefinite linin fibers. The spindles were successive, and all were followed by membranes developed from cell plates.

LEWIS (20), investigating *Riccia lutescens* and *R. crystallina*, found neither kinetic bodies nor multipolarity. No nucleolus appeared, and the chromatin massed excentrically in synizesis. Nuclear elongation established the poles of the spindle. There was a cell plate, but not reorganization of nuclei, nor interkinetic rest.

In *Riccia Frostii*, BLACK (3) found scant chromatin and linin, but there was a very definite nucleolus. The spindle arose from extra-nuclear material, and it developed an incomplete cell plate. None followed the homotypic spindles. Centrospheres were not found.

VON MEYER (21) found only a smooth chromatin nucleolus in *Corsinia marchantioides*, which roughened as it fragmented. Linin and chromatin connected freely with the nuclear membrane. An incomplete suspended cell plate after the first spindle was complemented by the secondary cell plates of the homotypic spindles. Spore protoplasts were cut out by their centiifugal growth. Later VON MEYER (22) found no very radical departure from this in *Plagiochasma rupestre*. The chromosomes were thought to be derived from a fragmented nucleolus.

GRAHAM (15) investigated *Preissia commutata*. A diplotene, presynaptic, continuous, chromatin-bearing spireme by diakinesis resulted in eight split, bivalent chromosomes. The nucleolus disappeared, giving rise (perhaps) to spindle fibers. The heterotypic spindle arose from hyaline polar caps, and it was followed by a cell plate. Reconstruction but not rest preceded two simultaneous, homotypic spindles.

Papers confirming reduction by successive bipolar spindles include: GARBER 1904, on *Ricciocarpus natans;* JOHNSON 1904, on *Monocloea;* HUMPHREY 1906, on *Fossombronia longiseta;* CLAPP 1912, on *Aneura pinguis;* MCCORMICK 1915, on *Symphogyna aspera;* FLORIN 1918, on *Chiloscyphus polyanthus*. Confirming quadripolar spindles are: CAMPBELL 1913, on *Calycularia radiculosa;* also 1914 on *Pallavicinia radiculosa* and *P. Zollingeri;* GRÜN 1914, on *Treubia insignis*. Subsequent to the brief observations of LECLERC DU SABLON in 1885, the only previous work on sporogenesis in *Reboulia* was that done by HAUPT (18) in 1921. In that study were discussed the general morphological details of the spore mother cells, the elaters, the tetrads, and the spores.

JUNGERMANNIALES.—Briefly to summarize the situation, we find the feature of the lobing of the spore mother protoplast to be undisputed. Excentric and lobed nuclei were reported only by DAVIS (8) and FLORIN (14). With the exception of some minor details, syni-

zesis or the first contraction is granted universally. Whether a true synapsis of parental spiremes occurs at that period is still debatable; FARMER heads the negative side. It is a matter of common agreement that diakinesis cuts out bivalent chromosomes from a chromatic spireme. The first separation is a transverse or reducing division (DAVIS 8, contra) in the sense common to sporogenesis in Spermatophytes. The second separation is a longitudinal or equational division. Whether there is complete, double, preparatory splitting of the chromosomes in prophase is a question that is rapidly being answered in the negative. The opposing school includes FLORIN and FARMER. The views of the latter are based upon the concept of a quadripolar spindle, typical for *Pallavicinia*. This structure is not given more than transitory value by more recent investigators. Most research indisputably reveals the presence of successive spindles in meiosis, but much variation is reported in the phenomena of interkinesis. It is evident that the terms "cell plate," "resting period," and "reorganization" need a more precise definition. The prevalent verdict is that rest, reorganization, and incomplete septa mark the period of interkinesis.

As regards kinoplasmic features, it is generally thought that a special zone comes to surround the nucleus in prophase. Its premetaphase manifestations are generally described as being of the independent multipolar type common to Pteridophytes and Spermatophytes. The only investigator to see specialized kinetic bodies in spore mother cells was FARMER (11). Centrospheres accompanied by centrosomes (*Pellia*, *Pallavicinia*, and *Fossombronia*) were found both here and in sporogenous and vegetative tissues. CHAMBERLAIN (6) and DAVIS (8), working with *Pellia*, found marked centrospheres in the germinating spore. Confirming this evidence for the presence of central bodies, FLORIN (13) has recently reported distinct centrosomes and asters in *Riccardia* (*Aneura*) after pairing of the pronuclei.

That all these bodies govern spindle formation and activity is granted. They are thought to arise in the cytoplasm *de novo*, and usually independently of each other. Together with the kinoplasm, they resolve into undifferentiated cytoplasm at the end of each mitosis. The chromosome count is becoming standardized at eight

for the gametophyte. The acrogynous Jungermanniales have still to be investigated.

MARCHANTIALES.—Barring the early amoeboid condition noted by HAUPT (18), the spore mother cells are spherical, *Fegatella* excepted. FARMER (11) and Miss MCCORMICK (23) reported contrary findings. Prophase phenomena do not feature the quadripolar figure. The spindles are bipolar, and originate from multipolar foci.

Instead of the four simultaneous centrospheres observed in the Jungermanniales, FARMER (11) here reported but two in *Fegatella*. No other worker has reported them in this phase of ontogeny. GRAHAM (16) has reported true centrosomes in fertilization for *Preissia* and also in stages of the young sporophyte up to four cells. In *Marchantia*, IKENO reported centrosomes as a constant feature of spermatogenous cells. MOTTIER (26) and VAN HOOK (28) found them in the gametophore of the same plant. The gametophytic chromosomes number eight generally. The further history of reduction differs in no appreciable way from that of the Jungermanniales.

Introduction

In undertaking the cytological problems of *Reboulia*, there would be no expectation of finding kinetic bodies in its ontogeny, with the possible exceptions of during the period of fertilization and in the germinating spore. The question of the quadripolar spindle would be eliminated, since all other Marchantiales display a reduction effected by two successive and independent mitoses.

As regards the early intent to prove a series of developments in the kinoplasmic architecture of the cell, phylogenetically significant from the lower Hepaticae up through the higher Hepaticae, as based upon comparative phases of ontogeny in all the genera, it must be said that such attempt has not succeeded signally. The accumulating evidence points to uniformity, and not to interpretations of cyto-phylogenetic difference. Much work will be required to arrive at any worthy conclusion in this matter; accordingly no discussion of this topic will be undertaken in this paper.

In the sporogenetic history, matters upon which there exists a wide variation of opinion include the origin of kinoplasm; its subsequent organization; the source and disposition of the chromatin;

the organization of the chromosomes; the source, character, and mutual relationships of nucleolus and chromatin-reticulum; the character and location of metaplasmic bodies; the precedence and extent of cell septa, and their part in cytokinesis and spore wall formation. To discuss these matters is the chief purpose of this paper.

Materials and methods

The material upon which this study of the sporogenetic processes in *Reboulia hemisphaerica* (L.)Raddi is based, was secured from Miner's Gully, Illinois, about 140 miles west of Chicago. This is a gulch of considerable extent, and is situated in the unglaciated portion of Jo Daviess County. Fixation in the field was not always feasible, so that some material had to be brought to maturity by means of outside bedding.

Fixation in the field made on April 9, 1921, yielded no studies in the reduction division. Transferred material (fixed daily) yielded the first spindles on April 19. This probably antedated reduction in the field by several days. In 1922 the late spring deferred reduction in outside bedded material to May 10. In favorable seasons *Reboulia* undoubtedly has completed its reduction divisions by the end of April.

Fixation in the field gave the best results, although transferred material did well if the specimens were kept in a turgid condition up to the time of immersion. Excision of the sporophytes proved to be difficult, and was found to be unnecessary. Incision of the dome of the carpocephalum secured rapid penetration. Any aperture made in the sporophyte resulted in the loss of the spore mother cells. A quick plunge into 95 per cent alcohol sufficed to dissolve any superficial protective substances.

Persistent efforts failed to devise any successful method for the use of the osmic series of fixing fluids. No bleach was of any service. In synizesis, MOTTIER'S mitochondrial formula gave admirable results when stained with safranin in conjunction with salkind blue. CHAMBERLAIN'S formula for the germinating spore of *Pellia* proved usable when the spores neared maturity. Outside of these two periods, no recommendation can be made for the employment of osmic acid as a fixing agent in the reduction division of *Reboulia*.

The best results were secured from the use of the chromic series of fixing agents. Stock solution gave the most delicate fixation. Bouin's fluid gave brilliant pictures, but resulted in considerable cell enlargement.

The most abundant metaphases were found at 11:00 A.M. The actual meiotic processes of any given sporophyte are accomplished within a very limited period, probably less than two days. To secure post-reduction phenomena required fixation for a much more prolonged period. The chloroform-paraffin method was used. Sections were cut 2–20 μ thick. Seven μ afforded the most serviceable material for the general study of cytological detail.

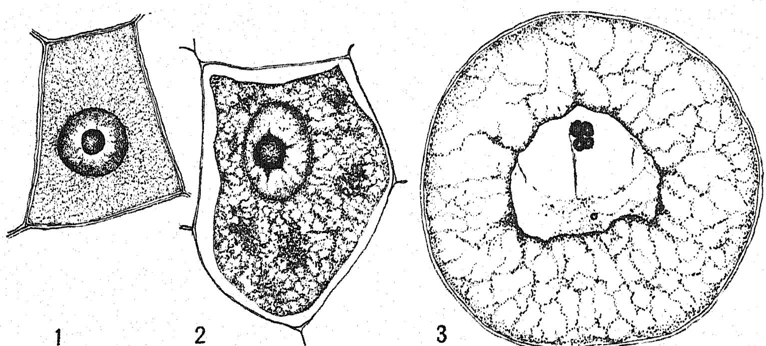
The inability to employ osmic fixation made the task of securing an adequate stain a difficult one. Iron-haematoxylin was not easy to employ because of the avidity with which the cytoplasm absorbed it. The chromatin also showed a tendency to accumulate the black pigment, and it was not retained well in the process of destaining, as the cytoplasm unfortunately held the haematoxylin much longer than did the chromatin. Mitochondrial stain proved most inapplicable. Flemming's triple stain gave satisfactory results, but the period in safranin had to be prolonged to two days; that in gentian violet to four hours. In sections thicker than 5 μ, where background was desirable, alcoholic light green proved to be a most effective substitute for Orange G.

Results

This study starts with the resting condition of the last sporogenous division. Figs. 1 and 2 represent early sporogenous cells from separate receptacles on a single thallus, the anterior one (fig. 1) bearing final sporogenous tissue, and the posterior (fig. 2) early meiotic tissue. Subject to the same growth environments, the same fixation, and the same stain on a single slide, they furnish authentic and comparative data. The sporogenous mass was a definite tissue, ellipsoid in transverse section, and measuring roughly 12 by 16 cells in its broadest diameters. Having used mitochondrial fixation (MOTTIER) and stain (WILSON), the tissues were so loaded with metaplasmic elements that finer details were obscured; but the vegetative tissues revealed that the action of the fixing fluid had been gentle. Fig. 1 shows the distinct walls of the sporogenous cells, and the dense

cytoplasm firmly inclosing the large spherical nucleus. The latter was turgid, and it contained a large, orbicular, deeply staining nucleolus. The linin, with extremely fine chromatin granules staining blue, appeared as a very tenuous film at the nuclear border, the nucleolus being centered within a broad hyaline areola. These nucleoli gave the tissue a sharply punctate appearance.

The honeycomb character of the tissue can be appreciated better from fig. 2. This represents a cell from the older and posterior sporophyte, in which considerable expansion had occurred without any



FIGS. 1–3.—Fig. 1, final sporogenous cell, resting condition; fig. 2, similar cell exhibiting meiotic prophase activities of growth and retraction from sporogenous cell wall; fig. 3, new spore mother cell wall; protoplast shows marked changes in size, form, and cytoplasmic arrangement; same magnification as figs. 1 and 2.

increase in the number of sporogenous cells. Near the center of the sporogonium the protoplasts had begun retracting from their sporogenous walls, which no longer stained as deeply nor appeared so thick as before. The continued stretching of these walls, induced by the progressive growth of the sporophyte, alters their angularity until the elongation of the elater cells ruptures the whole fabric. However, highly significant changes had occurred in the spore mother cell as the cytoplasm, replete with deeply staining metaplasm, had become vacuolated and coarsely stranded. This change indicated preparation on the part of the protoplast for the immediate elaboration of the future wall of the spore mother cell. This wall seemed to be created by an effusion of substance elaborated by the multitudinous granules of the peripheral portion of the proto-

plast. Capsular enlargement obtained progressively throughout the period of wall formation, but the mother wall thickened so rapidly that the reorganizing cells kept in close contact with the original sporogenous cell walls. Under suitable stain, this investment of diffuse opaque material about the spore mother protoplast was seen to be very thick, and it was much laminated. Subsequent to the amoeboid period (HAUPT 18), the rounding and enlargement of the protoplast caused this mother wall to become tenuous.

Before the protoplasts had retracted very perceptibly from their sporogenous walls, the nuclei betrayed presynizetic activity (fig. 4). The stainable chromatin collected into a single body in the midst of a mass of indistinguishably intricate linin fibers, which stretched away from the nuclear membrane to which they seemed to be connected by many unbroken filae. Fig. 5 shows complete synizesis. The massing of the synizetic figure occurs, very commonly, opposite a thickened bed of cytoplasm, but not invariably so. Whenever the synizesis juxtaposes a thick border of the protoplast, a condition indicating some sort of polarity of the entire cell is made very evident. In fig. 5 the nucleolus is uppermost in the knot, where it is crowding the linin to the nuclear wall. It is seldom that the figure is seen in any other position, that is, in any position that cannot be accounted for by the vicissitudes of orientation in sectioning. When the synizetic skein unfolds, the nucleolus will retreat in advance, and will pass ultimately to (or nearly to) the opposite wall. This corresponds with HARPER'S (17) findings in Ascomycetes. The nearly spherical form and the remarkable size of the nucleolus at this stage provide a strong argument for the presence of some osmotic or retention membrane at its periphery. Its absorptive power is indicated by its chromatin storage function. The linin beneath it shows a circle of strands much coarser than the fibrils of fig. 4. There is considerable evidence that the continuity of the presynizetic network is being somewhat broken up by the contraction.

When the nucleolar retreat ensues (fig. 6), the linin is drawn toward the antipolar border of the nucleus. It maintains its connection with the polar nuclear border by means of persistent anastomosing threads. This incipient spireme displays the coarsely outlined stranding of fig. 5, and certain irregularities suggest the localization

of the chromatin and the presence of anastomoses. Aside from a slight decrease in the size of the nucleolus, at this stage there is little to suggest the conventional spireme; nor, in the present series of preparations, has any evidence been furnished that a true synapsis of duplicate spiremes has occurred at any period.

The recovery from synizesis often displayed a course somewhat different from the behavior depicted in fig. 6, in that the linin fibers show more distinctly. The thread may be smoother, more definitely contoured, more chromatic; and it may show even more plainly its filar anastomoses with the nuclear wall. The nucleolus, however, will have diminished markedly in size (fig. 7), and it will show broad trails down which the chromatin seems to be passing to the linin reticulum. The central mass of the nucleolus, however, generally displays smoothness of contour. In fig. 8 the nucleolus has been bisected, each portion carrying its own quota of the spireme. This is as extensive separation of the nucleolar elements as would commonly occur, since by far the majority of instances show the nucleolus as a single body. Fig. 9 shows an unequal nucleolar division, attended by an inequality in the distribution of the spireme. This latter structure has lost the slightly beaded appearance of fig. 8, and it has diminished in number of internal strands, but peripherally there appear the cut ends of other strands abutting the nuclear membrane. How intimate may be the association of spireme and confining nuclear membrane is difficult to determine. This section was cut transversely to the polar axis of prophase.

Whether the nucleolus remains solitary, double, or quadruple in mid-prophase, and whether the spireme shows marked disassociation or not, there is always a very marked diminution in the amount of chromatin remaining visible within the confines of the nucleus. It is not thought that the linin threads of the spireme actually disappear, but their inability to retain stain causes them to simulate such disappearance. Such a suggestion is given by fig. 7, where little superficial chromatin remains on a spireme that has become a weft of linin fibrillae with conspicuous attachment to the nuclear border.

The cell now shows a period of rapid growth, and the intake of nucleoplasm may account for the irregularities soon supervening in the linin association. Without losing its connection with the central

chromatic mass, it becomes a diffuse tangle of threads that course erratically throughout the nuclear cavity (fig. 10). The figure is a presentation detailing nuclear phenomena as faithfully as possible. The disappearance of the chromatin is a problem, since the linin fibers are not visibly increased in number or in staining capacity. If it is resolved into karyolymph, this would account only partially for the marked nuclear enlargement. A marked increase which now supervenes in the staining power of the cytoplasm could hardly be correlated with the dissolution of the chromatin, unless there is partial exosmosis of the latter substance. This loss of visibly staining chromatin from the nucleus, however, is final and relatively complete. The ultimate breaking up of the nucleolar mass results in numerous very dense elements imbedded in an opaque matrix which is the source of the radiating meshes of the linin structure. The latter is so much reduced that it can hardly be thought of longer as a spireme.

As the cell approaches very closely to the actual reduction process, it becomes increasingly evident that not the linin spireme but the nucleolar body is the significant factor in the chromatic history of the cell. There is no indication of a segmenting spireme in the usual cytological sense, but there has been increasing evidence that surplus chromatin is being passed along to other places, the linin thread being but one of the means of translocation. Figs. 11–13 show that chromatin elision carried to its maximum extent leaves a residue, the metaphase chromosomes. Contrasted with the huge nucleolus of synizesis, the chromosomes are incredibly small, and they seem to emerge from nodal or specially localized points in the nucleolar mass.

At this period certain significant phenomena have occurred in the cytoplasm also. The early, densely granulose protoplasm has become a grossly vacuolated, mature cytoplast, composed of coarse radiating wefts of finer fibrillae. The plastids dispersed in its meshes are usually rendered invisible by the chromic fixation. Furthermore, great vacuoles in the protoplast cut out a dense layer of cytoplasm that invests the nucleus closely. This region finally becomes so reduced in thickness by the expansion of the nucleus that it becomes little more than an investment where all the peripheral strands of the

cytoplast terminate centrally. No evidence could be established that this zone constitutes a specialization in the form of kinoplasm, but it certainly represents the region in which kinoplasm would be expected.

The wefts of the cytoplast are radiating, infrequently anastomosing paths between two very significant regions, the periphery of the nucleus and the periphery of the cell. The exterior limiting layer has already functioned in laying down a protective wall, to which it is closely adherent unless disturbed by some extraneous interference. It is active as a secretory region, and undoubtedly has much control over nutritional elements entering from the carpocephalic tissues. It is a medium for a vacuum-like support of the protoplast, tending to prevent collapse with its attendant injury to the fine internal mechanism that is in control of cell metabolism. It is then not inconceivable that the inner layer investing the nucleus is also just as significant and possesses just as much control over the activities of the more central regions of the cell. Externally, a membrane was elaborated. Internally, there might appear (at a slightly different period of ontogeny) a slightly altered substance, kinoplasm, differing from the nuclear membrane itself mainly in the matter of continuity and cohesion. This secretory function conceivably might lie dormant, awaiting appropriate stimulus from some element not apparent as yet.

Shortly subsequent to synizesis, there appear within the nucleus certain bodies unfamiliar to the literature of reduction division. For convenience of terminology, they may be designated as nuclear bodies, since their activities are confined largely to that region of the cell. As their presence in the last sporogenous cells has not yet been established, for the purposes of this study it may be said that they arise during the early prophase of reduction division.

In character they are constantly small, homogeneous, brilliantly staining corpuscles; they are smooth surfaced, and apparently are inclosed in a thin pellicle of like character with the linin. This investment is hard to detect except under favorable differentiation. When seen it is quite as definite as the pellicle of a young plastid. What their origin might be was hard to determine; but the *locus* of their appearance usually was the region of the synizetic knot, sub-

sequent to some considerable alteration in the size, form, continuity, or position of the nucleolus. They cannot be interpreted casually as mere fragments of the nucleolus, as they appear long before that structure has resolved itself into granules approaching anything like a similar size. To follow their history serial sections are obligatory; sections 12–20 μ thick are best in very late prophase.

As a rule, the nucleolar bodies take the same stain as does the nucleolus. Under heavy counterstain they sometimes emerge in a contrasting hue, this phenomenon being due, undoubtedly, to the selectivity of their hyaline investment. Their apparently inconstant features are relative position, degree of prominence, and something of a variation in size. Very frequently one or both may be removed in sectioning.

The first appearance of the nuclear bodies was at the polar margin of the nucleus, and at the base of the unfolding spireme. In this obscure position they are inconspicuous; in later stages they are revealed prominently (fig. 7). Fig. 8 shows them obscured within the loops of the spireme. The nuclear bodies leave the synizetic knot very early, and pass to more or less opposite regions of the nuclear cavity (fig. 7), carrying with them certain attached aggregations of the linin. Sometimes they show discrepancy of size, but usually there exists the uniformity displayed in fig. 7; and especially is this the case in the stages nearing metaphase, when the mother cell has probably reached its maximum prereduction dimensions. Unless plasmolyzed, the nucleus has become so large and so symmetrically round that the cell presents a very striking appearance. After this period the nucleus begins to show irregularity, and its membrane becomes more difficult to detect. The nuclear bodies of the polar region are connected by linin threads to the remnants of the nucleolus, out of which are developing the chromosomes in the antipolar region of the nucleus (fig. 3).

When the bodies arrive at the exterior margin of the nucleus, they attach themselves to the nuclear membrane. A fluff or haze of a peculiar type begins to surround them, and it involves the adjacent cytoplasm. It appeared to be quite certain that the nuclear membrane disappeared first in their vicinity; the remainder passed away gradually. The nuclear angle in which the chromosome com-

plex rests is not an especially active center, as is evidenced in fig. 12; while the regions in which the nuclear bodies lie are most especially so. Sometimes the bodies are not so remote from each other, but they proceed to separate (fig. 11) until they establish a polarity of the protoplast diametrically opposed to the initial nuclear polarity. In the process the nucleus becomes very sharply angled at the points at which the nuclear bodies occur (fig. 11), while the remaining regions depart more slowly from the original rounded form. These changes result in a structure somewhat comparable with the early quadripolar figure so much discussed in the Jungermanniales. Any degree of plasmolysis at this period enhances the quadripolar effect by sharpening the natural outpocketings of the nuclear cavity.

When the nuclear bodies have access to the cytoplasm after the destruction of the nuclear membrane, they appear to become ensconced in its meshes, and they may become the center of an indefinite granular mass. Figs. 11–13 show a somewhat definite sequence in the behavior of these bodies as they exist in the border of the cytoplasm, and as they continue to move or to be drawn apart. Fig. 12 especially shows the major portion of the nuclear wall destroyed, and it portrays also the peculiar thickening and shaping of the cytoplasmic reticulum (upper pole) around the migrating nuclear body. The opposite pole was sectioned too close to the related body to show any special cytoplasmic aggregation.

Heterotypic spindle

The actual rise of the spindle was not demonstrable; but a prolonged checking up of data item by item, for previous and for subsequent periods, compelled the belief that these are the initial visible phases in the rise of the heterotypic spindle. This belief is fortified further (fig. 12) by the presence of the peculiar granulations that appear to the left of the chromosome group; these are thought to be the transected fibers of an early amphiaster. This would mean that spindle fibers begin to appear before the poles are diametrically opposite. Thus the earliest form of the spindle would be triangular, the poles developing from the polar nuclear regions, while the third angle contained the less active chromosome complex of the antipolar region.

Fig. 13 shows the spindle with a straighter axis, a vanished nuclear membrane, and a further emergence of the chromosomes. Although spindle fibers are not demonstrable, it is felt that they are being developed rapidly. The upper pole of fig. 12 has pushed farther into the cytoplasm, encysting itself, and great wefts of the cytoplasm are organized about it. The impression is gained that the poles are constricted cylinders of tough thick fibers mutually supporting and being supported by the jacketing cytoplasmic reticulum.

Difficulty was encountered in demonstrating certain continuous peripheral fibers which lay far outside the zone of the chromosomes in the metaphase plate. In fig. 14 they were secured by staining heavily with haematoxylin, with total omission of all destaining. It is thought that their relationship to the metaphase plate might indicate that some of these rather coarse fibers might have been derived from the nuclear membrane. At a later stage it is certain that other fibers, which end freely in the peripheral cytoplasm, come to lie exterior to these fibers. In fig. 14 the underlying equatorial plate, seen in profile, is so compact that the individual chromosomes cannot be distinguished.

The spindle is a structure of progressive development. Likewise its activities are discontinued, and its architecture is remodeled or dismantled just as gradually. In every direction from the pole, peripheral fibers continue to be sent to the outlying cytoplasm. They are not (as they have appeared to many observers) mere aggregations of the ordinary cytoplasm of the cell, but are gossamer strands inconceivably tenuous and smooth. They cannot be detected until they are present in abundance, and they are most visible *en masse*. Starting from a common center at the poles, numerous coarse fibers are seen to be enmeshed among much finer fibers. With regional restrictions, these latter permeate the protoplast, ending quite demonstrably in some strand of cytoplasm, a granule, or a plastid. These structures are definite asters; but they are so dense a complex of cytoplasm, kinoplasm, and trophoplasm that all attempts to detect the presence of any possible central body have proved fruitless. Such a structure as is shown in fig. 12 was too indefinitely delineated to warrant its classification as a central body. That these regions are a complex of differing protoplasmic gels is shown by the fact that

the spindle fibers and asters commonly take the violet tone of FLEMMING's triple stain, while cytoplasm and granulose kinoplasm are distinctly roseate under the same treatment.

There is little evidence for the presence of distinct mantle fibers, but there are many central fibers in the newly formed spindle. It is upon the outer fibers of this group that the chromosomes seem to be borne; indeed fig. 15 would indicate their presence throughout the group. Many times the chromosomes are so obscured that they would seem to be attached to the inner faces of the fibers upon which they are adherent. In anaphase it is these connecting fibers which transect, and which carry the chromosomes poleward. Such fibers of the group as do not bear chromosomes then develop, apparently, the usual double equatorial line of granular swellings that constitute the early cell plate.

The completed heterotypic spindle occupies the former nuclear cavity in such a way that it constitutes an intra-nuclear spindle, with its poles only imbedded in the cytoplasm. During the metaphase the spindle begins a period of elongation that so stretches the outlying cytoplast that it comes to envelop the spindle closely. The first indication of true metaphasic activity is a lateral extension of this taut spindle. The fibers spin outward at the equator until the densely packed chromosomes begin to separate laterally from one another. As the chromosomes separate, they appear (fig. 15) as 16 small, blunt rodlets, the bivalent chromosomes. No indication of the homotypic splitting exists in metaphase. Having been spread apart by the spindle fibers, the bivalent chromosomes continue to separate from one another (fig. 16) without displaying tetrad characters. Since in anaphase (fig. 17) more than 8 bodies pass toward each pole (14 having actually been counted), it is evident that the homotypic split, if present, is fully concealed. There appears to be no evidence either for or against telosynapsis. In late anaphase the polar group is very dense (fig. 18), and as it passes into telophase it is drawn very far toward the cell's periphery. When the kinetic pull is relaxed, and when reconstruction supervenes, the daughter nuclei settle back toward the former nuclear border.

From the time of the inception of the metaphase, the connecting fibers of the spindle have steadily spread outward in the equatorial

region, enlarging what was originally the nuclear cavity. At first this orbicular space is bounded by the spindle fibers which had pressed back the cytoplasm. The phragmoplast thus created probably incloses some karyolymph; it is most certain that the interzonal spindle fibers have contributed to the formation of an incipient cell plate traversing the phragmoplast cavity. The spindle fibers disappear, but the cell plate (fig. 19) persists. No evidence was afforded of the presence of a cellulose septum within it. Undoubtedly it is the first cleavage plane in the cell, but its complete functioning is delayed until after the time of the homotypic mitoses. Also the many peripheral fibers of the first spindle may have operated to extend the cleavage impulse beyond the confines of the phragmoplast, which evidently had terminated its possible fulfillment through the agency of the central spindle fibers.

Homotypic spindle

The interkinesis is brief, but the daughter nuclei approximate a condition of rest. The telophase group of fig. 18 becomes the object of a new diminution of chromatic matter. Nuclear and cell growth continue, and the cell may elongate very slightly before the final karyokinesis. The first hint of a membrane around the reorganizing nuclei appears as a thin blue halo whose borders follow the irregularities of the dense cytoplasmic wefts that held the heterotypic poles in position. The red staining chromatic body is inclosed. Small, deeply staining spherical bodies appear within the halo, and they usually keep in close contact within its borders. The chromatin masses break apart (fig. 19) and separate slightly. As they enlarge, the nuclei lose their irregularity; and if they have migrated far peripherally, they begin their retreat to the border of the persistent phragmoplast cavity. The nuclear chromatin then breaks up into successively smaller bodies, until it is well distributed upon a very evident slender linin reticulum. Several chromatin masses persist.

As the homotypic metaphase approaches, the nuclear bodies migrate to four distinct points upon the border of the former nuclear (now the enlarged phragmoplast) cavity. The nuclei elongate (fig. 20) as the nuclear membranes are fading, and this constitutes added evidence that the nuclear bodies free themselves from their im-

mediate nuclear confines, and follow along the kinoplasmic tracks derived from the disorganization of the peripheral fibers of the previous karyokinesis. What forces determine the axes and the terminal points of this migration is a feature of the cell's behavior that is not easily comprehended. Again, the nuclear bodies could not be identified with the poles of the spindles, which appeared more swiftly than had their predecessor, owing to the abundance of potential kinoplasm residuary from the heterotypic asters.

The homotypic spindles may be parallel, but usually they are opposed. Their formation draws or distorts the cavity of the phragmoplast, since they organize in part from the kinoplasmic substance of its borders (fig. 20). Slightly curved at first, they commonly straighten in anaphase because of the tenacious hold of their poles, which have become knitted solidly into the cytoplasm by means of their astral rays. With the exception of size, these asters are the duplicates of the asters of the first karyokinesis.

Sixteen bodies appear in the metaphase plate (fig. 21) which are interpreted as univalent chromosomes, since more than eight very small bodies pass to each pole in anaphase. At the poles the chromosomes collect into a ball as before, and the spindles elongate, thrusting the masses of chromatin well into the cytoplasm. Profuse peripheral spindle fibers again spread outward from each pole in a wide cone.

The extreme thrust of the telophase carries the four new nuclei to the periphery of the cell. Reorganization of the granddaughter nuclei begins almost at once, and its inception is evident long before the cleavage has appeared that actually divides the spore mother protoplast into four parts. As the nuclei retreat, a slight peripheral film again appears which becomes a rim or nuclear membrane whose irregularities straighten out and again inclose the diminutive nuclear bodies and the more perceptible masses of the chromatin. The latter break up into progressively smaller bodies, any residue becoming a new nucleolus bathed by an abundance of karyolymph. Finally, delicate anastomosing threads are visible that traverse the whole nucleus (fig. 23). With the completion of the quartering of the protoplast, the spore nuclei will have attained a normal spherical turgid-

ity, will be centrally located, and will not differ appreciably in size from the last sporogenous nuclei.

The present study does not support the view that the spore mother protoplast is divided by means of surface cleavage furrows. Both homotypic spindles complete their telophases by a peripheral extension of spindle fibers. Subsequent cell plates initiate two secondary cleavage planes which are opposed to the primary cell plate. While cleavage is effectual first at the center of the spore mother cell (fig. 22), it operates simultaneously and equally in all planes (fig. 24). The *modus operandi* is the appearance within the cytoplasmic strands of a duplicate bed of granulations that ultimately flow together to form cytoplasmic films, the plasma membranes. Later these are separated from each other entirely, by the severance of any remaining plasmodesmen. Stains afford no evidence of the presence of an intercalated cellulose wall (fig. 24) at this early stage.

Subsequently the segregated spores display denser cytoplasm just within the new plasma membranes; it becomes a very dense zone in which are held plastid primordia and other granules. The activity of wall secretion is begun previous to any very perceptible rounding of the protoplasts. These latter may not be in close contact centrally if the cavity of the phragmoplast has been large and persistent; but the rapid increase in cell turgor quickly obliterates this space, and the tetraplasts acquire ternate faces interiorly, and a convex surface exteriorly. The dense layers of cytoplasm just beneath the plasma membrane contain plastids in abundance, and they become much larger at this period. Oil globules and other metaplasmic granules are abundant.

This thickening of the exterior portion of each spore protoplast is so marked that it could be mistaken for an early manifestation of the new spore wall. It cannot be asserted, however, that the spore coat has begun to form at any time previous to the appearance of a faint thread of contrasting color between adjacent protoplasts. Later this thread widens into a broad opaque band which stains densely, and which thickens with equal rapidity between the ternate faces of the maturing spores, as well as on their exterior borders just beneath the mother wall (figs. 24, 25).

In fig. 26 the exospore is splitting. The exinium hugs the protoplast, while the perinium bulges marginally, preparatory to the growth of the basal flanges of the mature spore. Exteriorly (fig. 27) the perinium is thrown up into exaggerated excresences which are most marked on the outer convex surface of the spore. The underlying exinium shows numerous filar connections with the perinium. The thinner intine hugs the protoplast, and the underlying plasma membrane is very thin. The cytoplasm is replete with nutritive inclusions. The spore has increased in size enormously subsequent to the reduction division. This is shown in fig. 28, in which is depicted a normally ejected mature spore that has been reduced to one-fourth of the scale of enlargement that was established for this series of drawings.

Summary

1. The premeiotic protoplast shrinks away from its angular sporogenous walls. Within the space created it secretes a special external membrane that functions as the wall of the spore mother cell.

2. A marked contraction of the nuclear network is preliminary to the reduction division. This synizetic knot displays a large, orbicular, deeply staining nucleolus that rests at the polar border of the nucleus.

3. The nucleolus retreats from the polarized nuclear border toward an opposite antipolar region in the nucleus. Anastomosing freely with the nuclear membrane, the spireme recovers as coarse, looped strands centered in the nucleolus or in its disassociated parts.

4. There is no evidence of the pairing of spiremes, nor that the post-synizetic spireme is a continuous thread. Suffering constant elimination of chromatin, it is reduced to slender irregular strands.

5. The chromosomes are short thick rods. They are not derived by the segmenting of a visibly synaptic spireme, but arise as localized masses in the large chromatin nucleolus.

6. The sixteen bivalent chromosomes appear upon the early spindle in juxtaposition. They are disassociated in metakinesis upon reaching the equatorial plate stage. They show no evidence of tetrad structure, but split apart evenly upon leaving the metaphase plate; sixteen chromosomes arrive at each pole.

7. Reduction occurs by means of one heterotypic spindle succeeded by two simultaneous homotypic spindles that usually lie in opposed planes.

8. The homotypic metaphase plates show sixteen chromosomes; and as more than eight chromatids pass to each pole, the gametophyte is considered as having sixteen chromosomes.

9. An incomplete cell plate follows the heterotypic spindle. Further cleavage is deferred until the time of the homotypic telophase, when secondary cell plates cooperate in effecting a simultaneous cleavage of the mother protoplast in three directions. All originate as duplicate thickenings of the central spindle fibers.

10. Externally to the spindles, cytoplasmic rearrangement is continued under the control of peripheral spindle fibers. Cleavage proceeds centrifugally.

11. No intercalated septum occurs previous to general spore wall deposition. No ingrowth of the spore mother cell wall occurs, nor any furrowing in the periphery of the protoplast.

12. The spore coats are laid down in centripetal succession by the activity of the plasma membranes. The spores are freed by the rupture of the attenuated spore mother cell wall.

13. No specialized zone of kinoplasm, nor any hyaline polar caps were seen. The spindles possessed true asters.

14. Small intranuclear bodies seemed to have significance during the early stages of spindle formation. They showed no evidence of a centrosomic nature.

The writer is much indebted to the late Professor WILLIAM L. WOODBURN for valuable suggestions and criticisms in the development of this investigation.

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EXPLANATION OF PLATES XXX, XXXI

In the execution of these drawings a Bausch & Lomb 1.9 oil-immersion objective was used with a Huyghenian ocular 10. A camera lucida was employed at table level, with arm at 100, and draw tube of microscope at 160 mm. With the few exceptions noted, all figures were magnified 912 diameters. The plates were reduced two-fifths in reproduction.

PLATE XXX

FIG. 4.—Last sporogenous telophase, showing amoeboid protoplast retreating from tenuous cell wall.

FIG. 5.—Synizesis occurring at polar border of nucleus.

FIG. 6.—Recovery from synizesis by retreat of nucleolus from polar border of nucleus.

FIG. 7.—Antipolar position of nucleolus, showing chromatic linin strands attached to polar border of nucleus.

FIG. 8.—Early bisection of nucleolus, with linin associations attached to each segment.

FIG. 9.—Older stage; transverse view showing transected chromatin threads on nuclear border.

FIG. 10.—Nuclear details in section 10 mm. thick, showing attachment of linin thread to eroded nucleolus, nuclear border, and nuclear body: ×1140.

FIG. 11.—Orbicular nucleus changed to quadripolar figure, antipolar view; nuclear bodies situated in sharpened angles, with chromosomes lying at lower level.

FIG. 12.—Composition study showing adjacent astral and nuclear sections; cytoplasm being incorporated into astral complex; transected interzonal fibers at left of chromosome group; nuclear membrane still persists at right; nuclear body below.

FIG. 13.—Spindle axis established in one plane; nuclear bodies antipodal; nuclear membrane gone.

FIG. 14.—Heterotypic spindle whose peripheral connecting fibers wholly inclose equatorial plate.

FIG. 15.—Polar view of heterotypic metaphase plate.

FIG. 16.—Lateral view of very early heterotypic anaphase.

FIG. 17.—Later anaphase.

PLATE XXXI

FIG. 18.—Telophase showing thickening of spindle fibers in equatorial region.

FIG. 19.—Reorganizing daughter nuclei retreating from periphery of protoplast; phragmoplast cavity shows incipient cell plate.

FIG. 20.—Elongation of daughter nuclei indicating initial stages in formation of homotypic spindles.

FIG. 21.—Homotypic division showing opposed spindles; it presents one homotypic equatorial plate in polar view.

FIG. 22.—Completed homotypic anaphase, showing early nuclear phases of three associated spores; cleavage plane of first mitosis still visible.

FIG. 23.—Very late telophase, or formation of spore nuclei; cell plate significant of future cleavage lines.

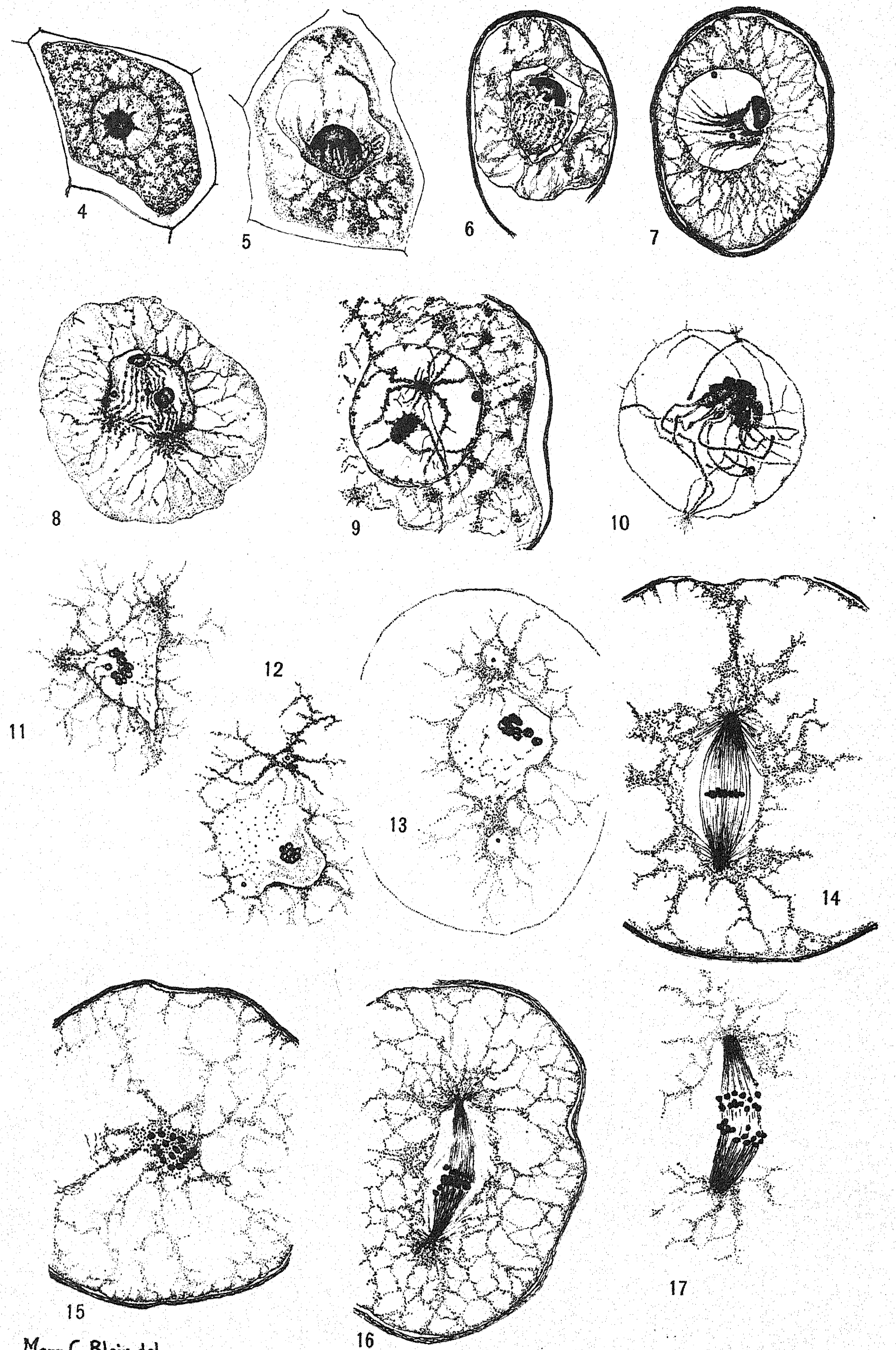
FIG. 24.—Early cleavage of mother protoplast, showing formation and progress of plasma membranes.

FIG. 25.—Exospore thickening around each member of tetrad; $\times 408$.

FIG. 26.—Exospore splitting into perinium and exinium; mother wall shown as ruptured remnant; $\times 408$.

FIG. 27.—Section of mature spore from black sporophyte just previous to rupturing of capsule; perinium folded; exinium thick; intinium invests plasma membrane; $\times 408$.

FIG. 28.—Outer face of ejected spore showing reticulations and marginal rim; $\times 207$.



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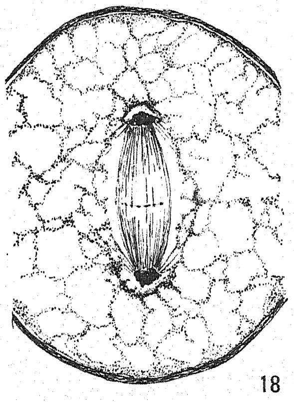

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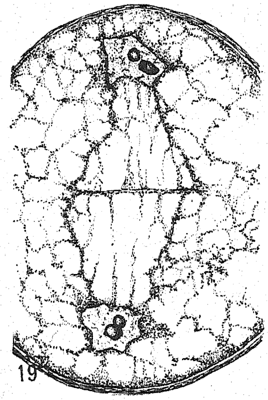

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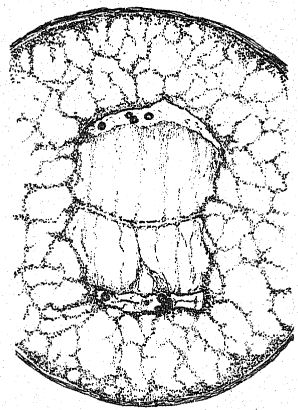

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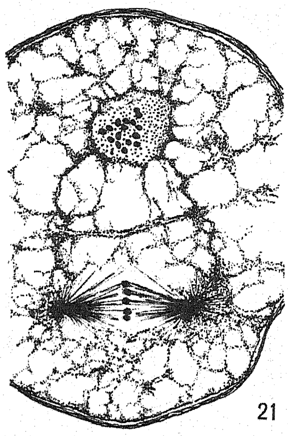

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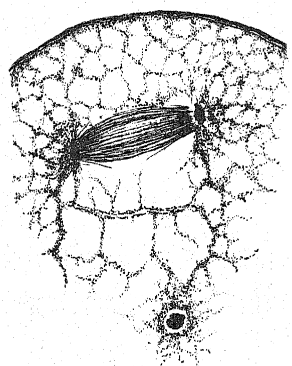

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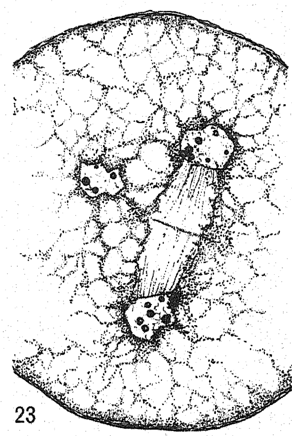

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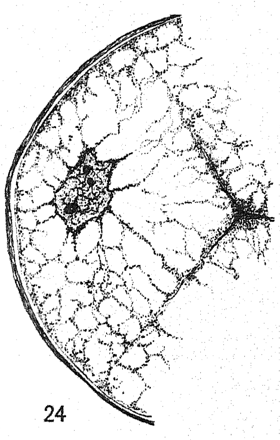

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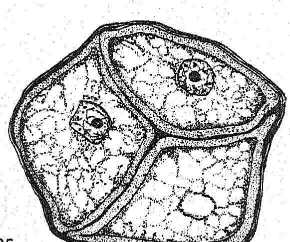

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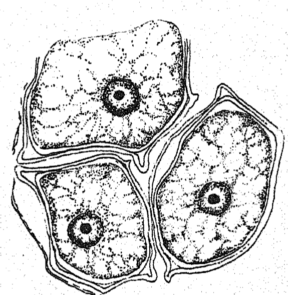

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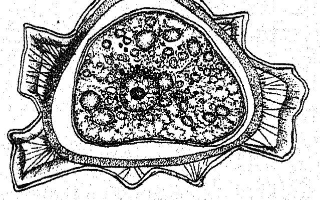

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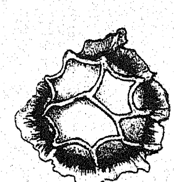

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HYBRIDS IN CYCADS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 348

CHARLES J. CHAMBERLAIN

(WITH THIRTEEN FIGURES)

It is doubtful whether any true hybrids in cycads have ever been described, although there is a note on hybrid cycads[1] dating back as far as 1883. In FOCKE'S *Pflanzen-Mischlinge* (1881), all the principal reputed plant hybrids are listed in systematic order, but no cycads appear on the list. He makes the statement, however, that the pollen of cycads "has occasionally been employed to fertilise the female of other species; but I am unaware whether perfect seeds have been obtained or hybrids raised."

In the note on hybrid cycads, HEMSLEY states as follows:

> Mr. KATZER, chief of the Imperial Gardens at Paullowsk, near St. Petersburg, had succeeded in raising a hybrid between *Ceratozamia longifolia* and *C. mexicana*. In this instance, a male of *C. longifolia* first developed an inflorescence, and Mr. KATZER collected the pollen and preserved it in a sealed bottle until a plant of *C. mexicana* produced a cone. When the flowers of the latter were in a receptive condition they were sprinkled with the pollen of *C. longifolia*, resulting in the development of perfect seeds, some of which germinated last year (1882).

These were claimed to be the first hybrid cycads; but as soon as this claim appeared, it was found that WEILBACH, Curator of the Botanical Garden at Copenhagen, had fertilized *Ceratozamia robusta* Miq. with the pollen of *C. brevifrons* Miq., and obtained seeds which germinated. These hybrids were sent to various gardens. MIQUEL himself determined the species of the parents; but WARMING later identified the so-called *C. robusta* as *C. longifolia*, and *C. brevifrons* as *C. robusta*.

HEMSLEY remarks:

> There is only one thing that is certain in all this, and that is the uncertainty of the so-called species. It is quite possible, and indeed very probable, that we have here in both instances to do with the male and female of the same species.

[1] HEMSLEY, WM. B., Hybrid cycads. Gardeners Chronicle 19:466-467. 1883.

A study of *Ceratozamia* in the field and in the greenhouse for a period of twenty years enables me to state with confidence that the uncertainty in those determinations is even greater than HEMSLEY intimated. DECANDOLLE, in his *Prodromus*, lists *C. longifolia* and *C. robusta* under *species dubiae*. They are more than doubtful. It is safe to say that seeds of a single cone of *C. mexicana* could be made to yield plants of *C. mexicana*, *C. longifolia*, *C. robusta*, *C. Miqueliana*, and *C. brevifrons;* and this result could be brought about by raising the plants in containers of various sizes and by planting in the ground in various conditions. The different characters could probably be developed in less than twenty years.

My principal study of *Ceratozamia* in the field was made near Jalapa, Mexico, on the steep, well drained mountain side opposite the extinct crater of Naolinco. All the plants seemed to be typical *C. mexicana*. Plants raised from seeds collected here and planted at the University of Chicago were easily diagnosed as *C. mexicana;* but after fifteen years many of them had become *C. longifolia*. None appear to be *C. brevifrons*, but that may be due to the fact that none of them were kept in small containers. *C. Küsteriana* may be a good species, but it looks as if the rest could be raised from *C. mexicana*.

The pollen of cycads is short lived. It gives the greatest percentage of germination when first shed; after a few days there is a notable decrease in the number of spores which germinate; at the end of ten days less than half the spores germinate; at the end of three weeks very few spores germinate; and a month from the time the pollen has been shed I have not been able to germinate the pollen of any cycad. Mrs. ALICE BAILEY, who is particularly skillful with culture media, was able to germinate pollen a few days after my attempts failed, but a month can be regarded as the limit of life of *Ceratozamia* pollen.

This would mean that while KATZER went through the motions of pollination, it is practically certain that there was no fertilization. That embryos were developed and seeds obtained I do not doubt; but it is well known that in *Ceratozamia* and *Encephalartos* the female cones and their seeds develop to full size, even without any pollination. SEDGWICK[2] proved conclusively that *Encephalartos* sometimes

[2] SEDGWICK, P. G., Life history of *Encephalartos*. BOT. GAZ. 77:300–310. 1924.

produces embryos without any pollination. There is, in this case, a fusion of the ventral canal nucleus with the egg nucleus, just as one finds it occasionally in *Pinus*, *Picea*, and *Ginkgo*. It is practically certain that KATZER'S so-called hybrids were of this type, or at least were produced without fertilization by sperms. To summarize, it may be stated, as it was said in 1851 and 1882, that the plants here described are the first hybrid cycads.

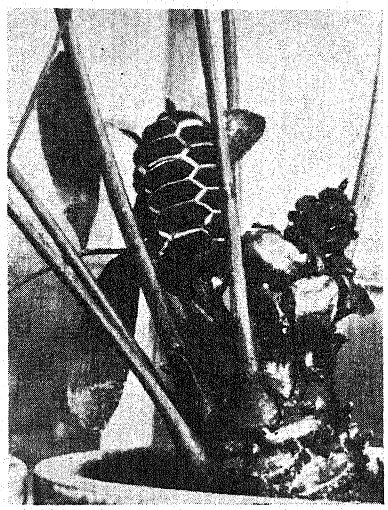

FIG. 1.—*Zamia latifoliolata:* cone at right, showing two of the ripe seeds, pollinated with *Z. pumila;* cone at left pollinated a year later with *Z. floridana*.

Even in limited collections in greenhouses there are occasional opportunities for crossing cycads. The pollination should be done just as the scales of the female cone have opened enough to allow the pollen to enter easily. When the opening between the scales is at its maximum, the pollination drop at the end of the micropyle is in the best condition for receiving the pollen. If pollen is abundant, it may be placed on a sheet of paper and puffed against the female cone; but if the pollen is from a small cone, like that of *Zamia latifoliolata*, a method devised by our head gardener is better. He takes a long glass tube, with one end drawn out like the tip of a pipette, touches some pollen with this tip and blows it into the crevices between the scales.

During the past five years several cross pollinations have been made in the University of Chicago greenhouse, most of them between different species of the same genus, but some of them between different genera. With the parents and the F_1 generation well labeled, it may be possible, within the next ten years to pollinate intelligently for an F_2 generation.

Zamia latifoliolata × Z. pumila

On December 20, 1921, I pollinated the female cones of two plants of *Zamia latifoliolata* with pollen of *Z. floridana*. The *Z. latifoliolata* was brought from Porto Rico, and the *Z. floridana* was secured from Miami, Florida. The scales of the female cone were

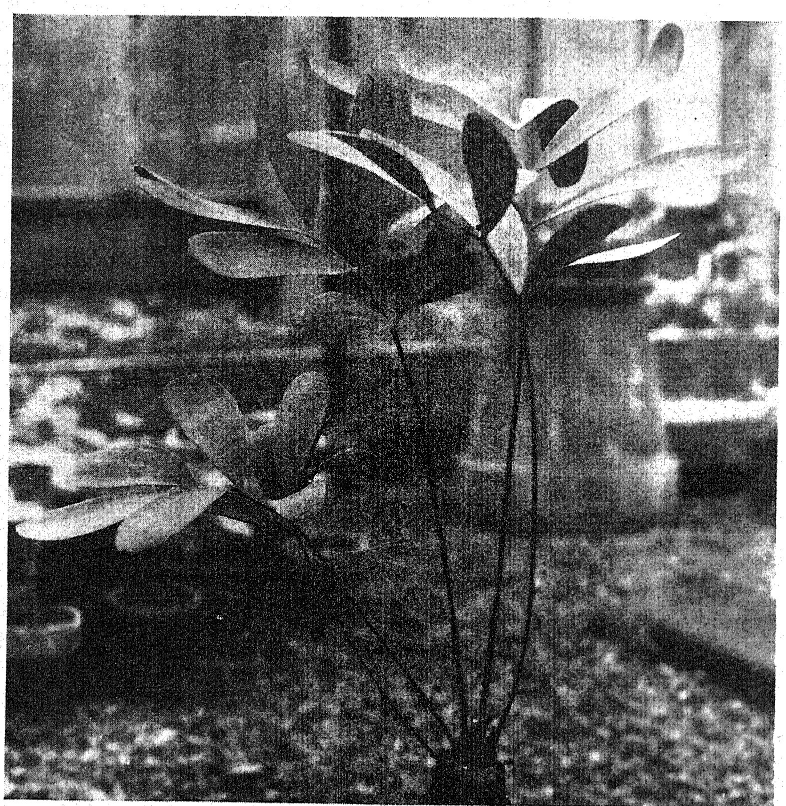

FIG. 2.—*Z. latifoliolata* × *Z. pumila*, F_1 generation

tightly closed, and it is doubtful whether any of the pollen not washed off by spraying survived until the scales opened. A week afterward the scales of the female cones opened and both cones were pollinated with pollen from a plant of *Z. pumila* which had been secured at Hawks Park, Florida. One of the cones produced three good seeds and the other produced four, all of which were

planted and four of which germinated. The condition of one of these cones when the seeds were ripe is shown in fig. 1. The cone at the left in this figure is a year younger. Its scales are just beginning to open. Two or three days after this negative was made, several of the scales were open and the cone was pollinated (December 15,

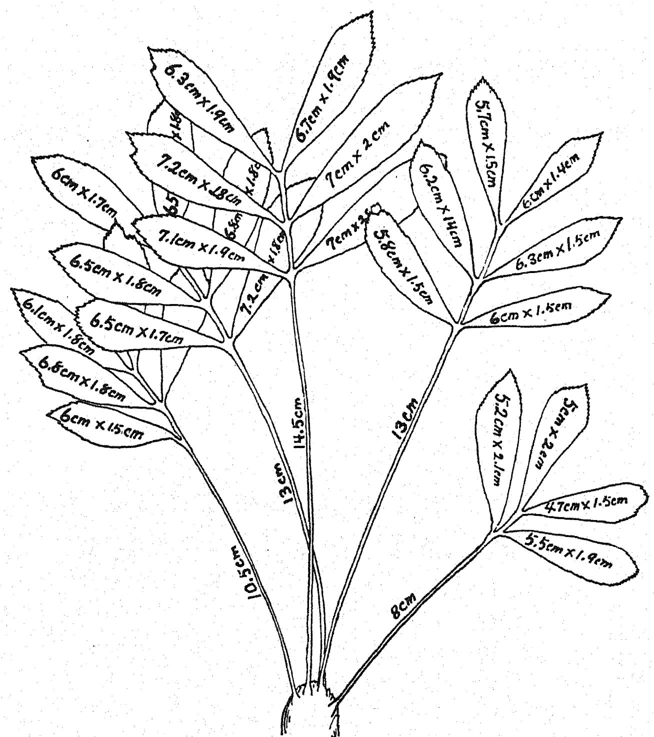

FIG. 3.—*Z. latifoliolata*×*Z. pumila*, showing size relations; one-half natural size

1922) with *Z. floridana*. This also seemed to be a successful pollination, for the cone grew, and on November 21, 1923, the seeds were ready to fall out.

The four seedlings resulting from pollination by *Z. pumila* grew vigorously, and on February 26, 1925, a little more than three years after the pollination (December 30, 1921), and two years after planting the seeds (January 9, 1923), they presented the appentrasts

shown in fig. 2. The size relations are shown somewhat diagrammatically in fig. 3.

It is evident that, in general habit and the character of the leaves, *Z. latifoliolata* is dominant, and that *Z. pumila*, which has comparatively small narrow leaflets, is recessive. The F_1 generation also resembles the dominant parent in the general contour of the leaflets, their serration, and in the number and branching of the veins.

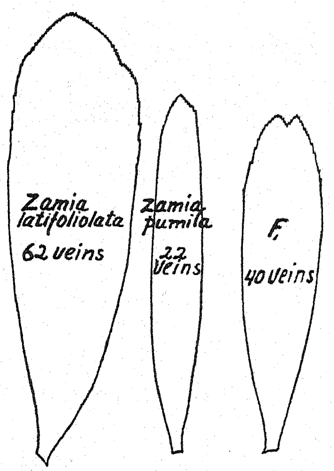

FIG. 4.—*Z. latifoliolata* × *Z. pumila*, leaflets of both parents and hybrid; one-half natural size.

The contour of the typical leaflets of *Z. latifoliolata*, *Z. pumila*, and their hybrid is shown in fig. 4. The veins are very numerous in *Z. latifoliolata*, often more than sixty; while in *Z. pumila* there are about twenty. In the F_1 generation the average number is about forty. We do not have seedlings of *Z. latifoliolata*, but four leaflets on a leaf developing from a bud scale had 33–40 veins. In the cycads such leaves resemble those of seedlings, or at least are juvenile in character. So it is probable that, so far as the number of veins is concerned, the F_1 generation closely resembles that of *Z. latifoliolata*. The histological character of the leaflet is also more like that of *Z. latifoliolata*, especially in the loose texture of the parenchyma between bundles.

If these four F_1 plants should reach the coning stage, as they may within the next ten years, and both sexes should be represented, and male and female cones should be produced at the same time, it would be interesting to see what would appear in the F_2 generation. Since the cones of the two parents are as distinct as their leaves, another pair of characters could be contrasted even before the F_2 generation is secured. Although *Z. latifoliolata* has a larger leaf, which is dominant in the hybrid, *Z. pumila* has a larger stem and three cones.

Zamia latifoliolata × Z. floridana

The cone of *Z. latifoliolata*, shown at the left in fig. 1, was pollinated December 15, 1922, with pollen from *Z. floridana*. The seeds were planted November 25, 1923, and four seedlings survived. Their appearance on February 23, 1925, is shown in fig. 5 and in the somewhat diagrammatic fig. 6, which records details of size relations.

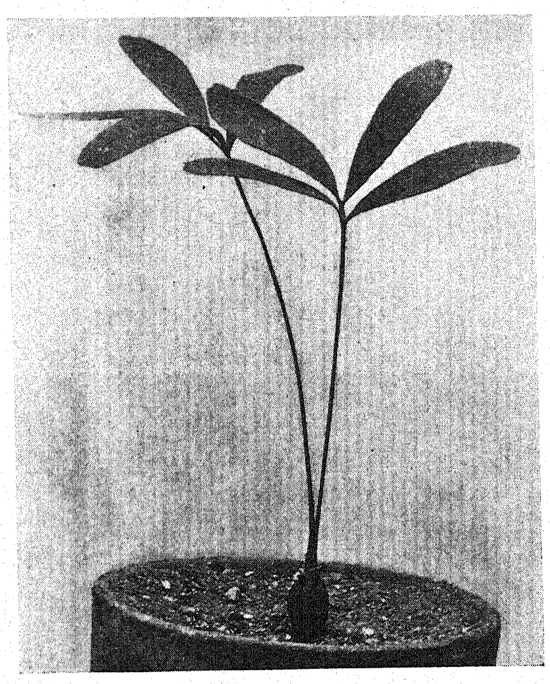

FIG. 5.—*Z. latifoliolata* × *Z. floridana*, F_1 generation

Here again it is evident that *Z. latifoliolata* is dominant and that *Z. floridana* is recessive. The seedlings look very much like those of *Z. latifoliolata* × *Z. pumila;* but it must be remembered that the female in this case is not only of the same species as that of the first hybrid described, but that it is the same plant. Besides, *Z. pumila* and *Z. floridana* are very nearly related species. The leaflets have the contour, serration, and venation of *Z. latifoliolata*. Fortunately in this case there are seedlings of *Z. floridana* for comparison (fig. 7). Its long narrow leaflet, with comparatively little serration, contrasts

sharply with the broad leaflet of *Z. latifoliolata*. Even the F_1 seedling shows double the number of veins found in the adult leaflet of *Z. floridana*.

In foliage *Z. floridana* differs more widely from *Z. latifoliolata* than does *Z. pumila*, and in the histological structure of the leaf the two species can be distinguished easily. In transverse section, the

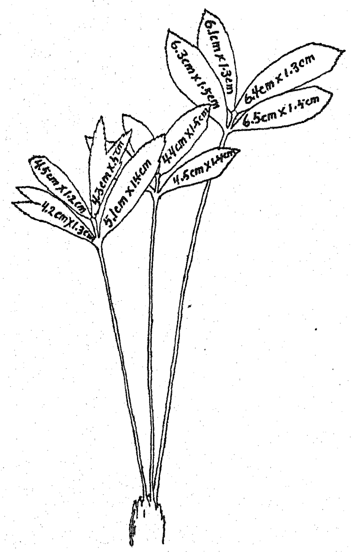

FIG. 6.—*Z. latifoliolata*×*Z. floridana*, habit sketch of F_1 showing size relations; one-half natural size.

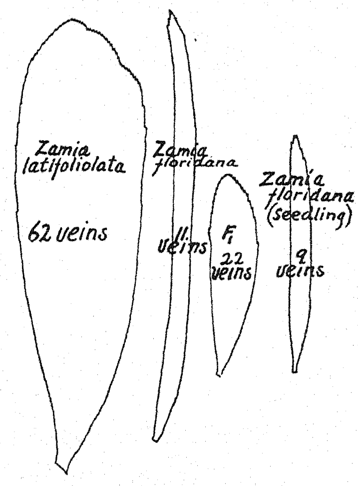

FIG. 7.—*Z. latifoliolata*×*Z. floridana*, leaflets of both parents and hybrid, also leaflet of *Z. floridana;* one-half natural size.

leaflet of *Z. latifoliolata* shows 6–8 suberized tracheids around the vascular bundle, while *Z. floridana* shows two or three times as many. The hypodermal layer of the abaxial side of the leaflet of *Z. floridana* is usually more or less sclerotic, while such a thickening is confined to the epidermis in *Z. latifoliolata*, except of course at the bundles, where all the cycads have sclerotic tissue.

The hybrid shows no sclerotic hypodermis on either side of the leaflet. The number of the suberized tracheids surrounding the bundle, as seen in the transverse section, varies from five to seven, while in the seedling leaflet of *Z. floridana* the number varies from eleven to fourteen. The mesophyll of the hybrid has large air spaces

like those of *Z. latifoliolata* and not at all like those of *Z. floridana*, in which this tissue is even more compact than in *Z. pumila*. In short, the foliage of the hybrid resembles that of *Z. latifoliolata*, both in topography and histological structure. Consequently, the dominant parent is the one with the larger leaves and broader leaflets.

FIG. 8.—*Z. pumila*×*Z. latifoliolata*, nearly mature cone

The cones and stems of *Z. floridana* are larger than those of *Z. latifoliolata*, but it will be about ten years before it will be known which parent is dominant in this respect.

Zamia pumila×Z. latifoliolata

On January 25, 1924, a female cone of *Zamia pumila* from Hawks Park, Florida, was pollinated by *Z. latifoliolata*. Only the upper

scales were open, but the cone grew to the usual size of cones of *Z. pumila*, and looked like a normal cone of this species (fig. 8). The first week in January 1925, the peduncle was accidentally broken, but the seeds were nearly ripe and the seed coats had an orange red color tinged with the darker red of *Z. latifoliolata*, rather than the light red with prominent orange which characterizes *Z. pumila*.

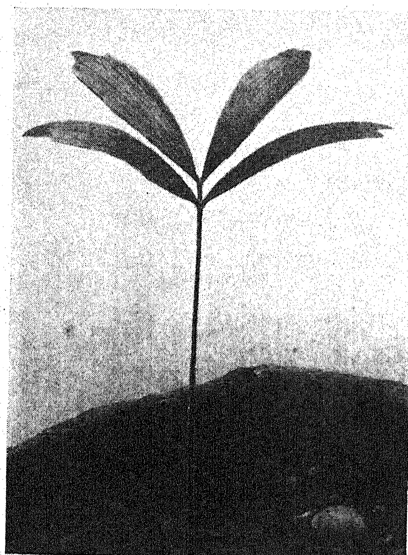

FIG. 9.—*Z. pumila*×*Z. latifoliolata*, F_1 generation.

The seeds were planted February 19, 1925, and several embryos broke the seed coat, but only one survived (fig. 9).

The color of the seed coat and the general appearance of the seedling are those of *Z. latifoliolata;* so that in this case, as in the reciprocal cross *Z. latifoliolata*×*Z. pumila*, the *Z. latifoliolata* is dominant.

Zamia latifoliolata × Zamia monticola[3]

On March 15, 1924, a cone of *Zamia latifoliolata* was pollinated with *Z. monticola*, and four days later another cone was pollinated from the same male cone. The female cones grew to the usual size of cones of *Z. latifoliolata* and the seeds seemed to be normal. The color of the seed coat in the first cone was somewhat lighter and with more orange than in the typical cone of *Z. latifoliolata;* but in the second cone the seeds had the dark red color characteristic of *Z. latifoliolata*, with scarcely any tinge of orange.

The seeds were planted late in December 1924, but as yet no seedlings have appeared. Although the seeds have not decayed, it is doubtful whether any seedlings will be secured.

[3] New species of *Zamia* described in BOT. GAZ. 81:218–227. 1926.

Ceratozamia mexicana × Zamia monticola

The most striking and successful cross made is one between *Ceratozamia mexicana* and *Zamia monticola.* One cone was pollinated March 23, 1924, with pollen from the same plant of *Z. monticola* which had produced the cones used in all the previous pollinations in which this species was one of the parents. Another cone of *Ceratozamia* on another plant was pollinated on April 25, 1924, with pollen from another cone of that same plant of *Z. monticola.* This new species is very favorable for hybridizing, because, during the period described in this paper, it has coned every year, producing from four to six cones at a time. The cones appear in succession, and shed their pollen in succession. Of the six cones which appeared in 1920, the first began to shed pollen December 14, 1920, and the last pollen from the sixth cone was shed February 9, 1921. Since each cone continues to shed pollen for about a week, there was a period of nearly two months in which any female cone, which might open its scales, could be pollinated. I have not had under continuous observation any other cycad in which the period of shedding pollen was so prolonged, but there can be no doubt that in *Macrozamia Moorei,* with its 20–100 males cones developing in spiral succession, the shedding period is much longer.

From these two cones nearly a hundred seeds appeared to be well developed. They began to fall out naturally November 27, 1924, and during the next few days all were planted, except a few which were reserved for dissection.

Ceratozamia is unique among the cycads in having only one cotyledon, while all the rest have two. This fact was noted first by VAN TIEGHEM,[4] and later confirmed by WARMING,[5] MATTE,[6,7] Sister HELEN ANGELA,[8] and others. MATTE,[7] however, found two cases in

[4] VAN TIEGHEM, P., Symetrie des structures des plantes. Ann. Sci. Nat. V. **13**:204. 1873.

[5] WARMING, E., Ein Paar nachträgliche Notizen über die Entwickelung der Cycadéen. Bot. Zeit. **36**:737. 1878.

[6] MATTE, HENRI, Note preliminaire sur les germination des Cycadées. Rennes. 1907.

[7] ———, Memoires société Linnéene de Normandie. **23**:35–94. 1908.

[8] DORETY, Sister HELEN ANGELA, The embryo of *Ceratozamia;* a physiological study. BOT. GAZ. **45**:412–416. 1908.

which there were two cotyledons. VAN TIEGHEM'S observations were made upon four "hybrid" seedlings secured by pollinating *C. longifolia* with pollen from *C. mexicana* which had been shed three years before the female cone was pollinated. In three of the seedlings there was only one cotyledon, but the fourth seemed to have two very unequal cotyledons. In germinating a large number of seeds I never happened to notice more than one cotyledon. Sister HELEN ANGELA examined more than a hundred embryos and every one had only one cotyledon. In microtome sections for an anatomical study, however, she noticed occasionally a few tracheids opposite the cotyledon. *Ceratozamia* is unique among cycads in another feature, the seeds drop out of the cone soon after fertilization and before the various regions of the embryo have differentiated. Cones were secured from Mexico shortly before the time for the seeds to drop out, and Sister HELEN ANGELA fastened a great number of them to clinostats which were rotated until the embryos were mature. All of the embryos on the clinostats had two cotyledons; but checks, planted normally, had only one. This was conclusive evidence that *Ceratozamia*, phylogenetically, had two cotyledons, one of which had become suppressed.

If the seeds planted by MATTE (some of which were of my own collection from the same locality which produced the seeds studied by Sister HELEN ANGELA) were planted in any other position than on the side, I should expect them to develop two cotyledons. We always plant the seeds of cycads with the long axis parallel with the soil and nearly but not quite covered by the soil. More than sixty seeds planted in December 1924 germinated. Without disturbing the young seedlings, one could see that many of them had two cotyledons. In July 1925, when the seedlings were being repotted, a careful examination was made, and forty-seven of the fifty-six seedlings which had survived up to this stage showed two cotyledons; three had one cotyledon, and in the other six the cotyledon situation could not be determined without sacrificing the seedlings.

The two cotyledons are shown in fig. 10, and also in *A*, *B*, and *C* of fig. 11. In *C* the embryo has been removed from the endosperm. The development of the two cotyledons proceeds as in other cycads. In the usual single cotyledon of *Ceratozamia*, the outline of the

cotyledon is often somewhat C-shaped in transverse section, so that by looking at the open side of the C, one might imagine that there were two cotelydons; but a section of the hybrid embryo shows the

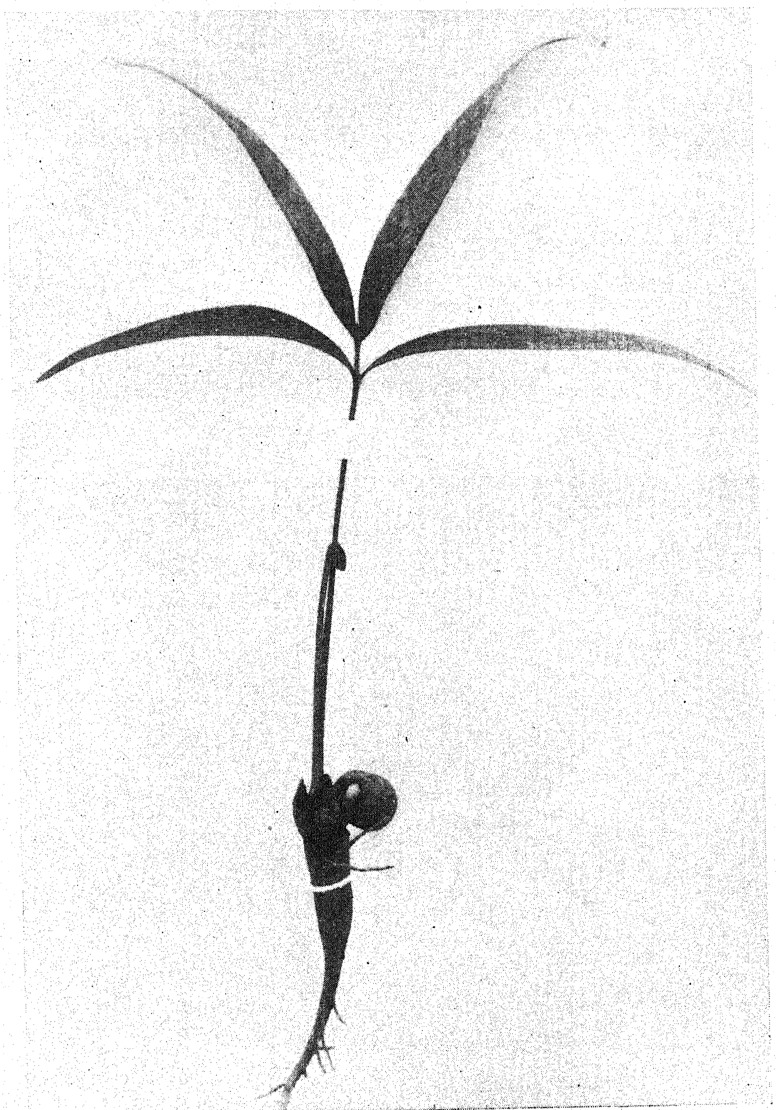

FIG. 10.—*Ceratozamia mexicana*×*Z. monticola*, F_1 generation

two cotyledons clearly, with the vascular supply just as described by Sister HELEN ANGELA for the dicotyl embryos developed on the clinostat.

One of the plants with a single cotyledon is shown in fig. 11 *D*. The stem apex lies between the leaf bud (*b*) and the cotyledon (*c*), but the suppression of the second cotyledon gives the leaf the position of the missing organ. Sections would show a few tracheids be-

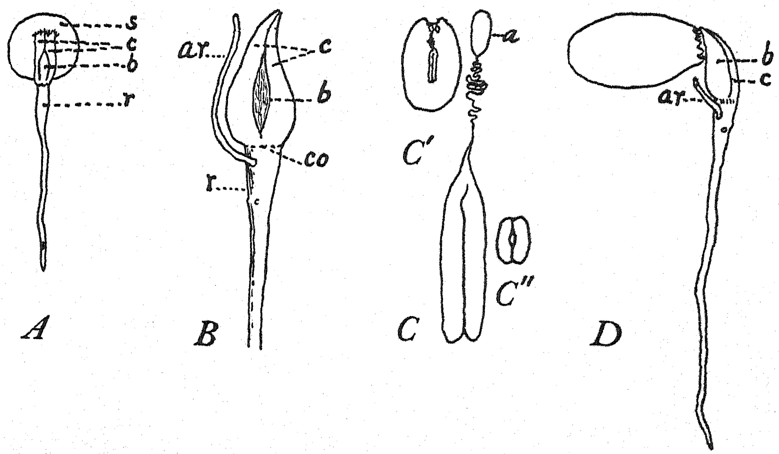

FIG. 11.—*Ceratozamia mexicana*×*Z. monticola*, F_1 seedlings: *s*, seed; *c*, cotyledons; *b*, bud; *r*, primary root; *ar*, apogeotropic root; *co*, coleorhiza; *a*, archegonium; *A*, *B*, and *C*, with two cotyledons; *D*, with only one; *C′* embryo in endosperm; *C″*, transverse section of embryo showing two cotyledons.

longing to the abortive cotyledon, so that the leaf bud is really between the two cotyledons. The apogeotropic roots, shown in *D* and also in *B*, are extremely common in cycads and are not at all the result of hybridization.

The leaflets of most of the seedlings are like those of *Ceratozamia* in contour, and all agree with *Ceratozamia* in having no serration; but many leaves of *Zamia monticola* have no serration. However, most of the leaflets are broad and have the contour of *Z. monticola*.

In this hybrid, *Zamia monticola*, the male parent is dominant in the character of the cotyledons and in most of the leaflets, while *Ceratozamia* is dominant in the general topography of the seedling and the contour of some of the leaflets. *Ceratozamia mexicana* has

a long slender leaflet tapering to a sharp point, and, like all species of *Ceratozamia*, if there is more than one species, entirely lacking in ser-

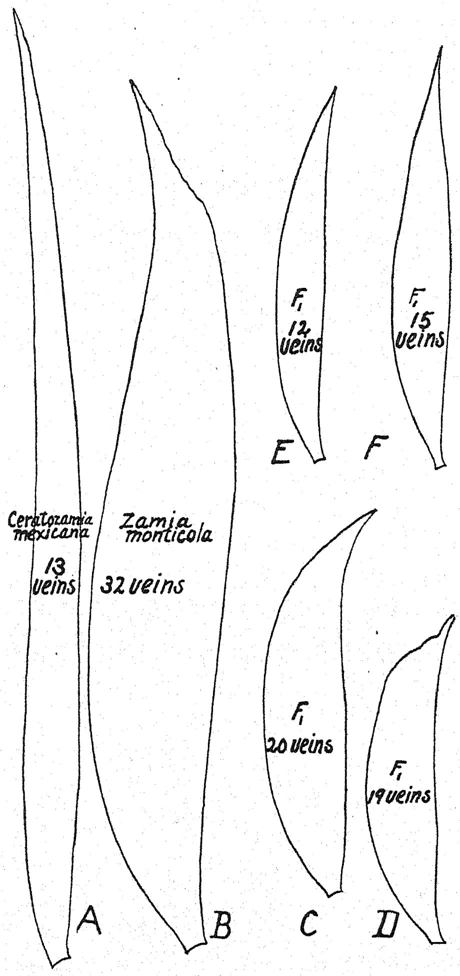

FIG. 12.—*Ceratozamia mexicana*×*Z. monticola: A, B*, leaflets of two parents; *C, D*, leaflets resembling those of *Z. monticola; E, F*, leaflets with contour of *Ceratozamia mexicana;* one-half natural size.

ration. In *Z. monticola* the leaflet is comparatively short and blunt at the apex. The contour of typical leaflets of the two parents and also leaflets of the hybrids are shown in fig. 12. In *C* and *D* of this

figure the leaflets have the contour of those of *Z. monticola*, and the number of veins is larger than in the adult leaflet of *Ceratozamia;* while in *E* and *F*, and also in fig. 11, the leaflets have the contour of those of *Ceratozamia*. The number of veins in leaflets of the *Ceratozamia* type, while smaller than in leaflets of the *Z. monticola* type, is nevertheless as large or larger than in adult plants of *Ceratozamia*.

FIG. 13.—*Z. pumila*×*Encephalartos villosus*, female cone shedding seeds

The histological structure of the leaflet of the hybrid resembles that of *Z. monticola* in the small number of suberized tracheids surrounding the bundle, and in the scarcity or entire lack of such tracheids between bundles. The difference here is very striking, because *Ceratozamia* has many such tracheids around the bundle and also between bundles. The mesophyll cells of the hybrid, in transverse section of the leaflet, are much elongated between bundles, while in *Ceratozamia* they are shorter and the tissue is more compact.

To summarize, the hybrid resembles *Z. monticola* in having two cotyledons and in the histological structure of the leaflet, as well as in the contour of most of the leaflets.

In this case both parents are large plants, with leaves more than a meter in length. *Ceratozamia* is arborescent, and this specimen of *Z. monticola*, a new species and the only specimen known, looks as if it might develop a trunk as large as that of *Ceratozamia*.

In the other crosses just described, all of them between different species of *Zamia*, both parents have small, tuberous, subterranean stems, and the parent with the greater display of foliage has been dominant in leaf characters.

In the cross between *Ceratozamia mexicana* and *Zamia monticola*, the latter is dominant in the characters of the leaf and cotyledons. The female cone of *Z. monticola* is not known, but the male cone, while very large for a *Zamia*, does not reach half the size of the average male cone of *Ceratozamia*. With about fifty hybrids now growing vigorously, it is quite possible that within nine or ten years there may be cones of both sexes, so that the cone character may be determined and an F_2 generation obtained.

Zamia pumila × Encephalartos villosus

On December 12, 1924, a female cone of *Zamia pumila* was pollinated with *Encephalartos villosus*. The *Zamia* was transplanted from Hawks Park, Florida, in 1914; the *Encephalartos* was secured from the Botanic Garden at Durban, South Africa, in 1912.

Only the upper scales of the female cone opened, but the pollination seemed efficient, for the cone grew and attained the usual size of cones of *Z. pumila*, instead of degenerating when not pollinated, as do the female cones of all the species of *Zamia* described in this paper. By the middle of October, 1925, the seeds were breaking loose from the sporophylls, and two weeks later the cone fell apart and the seeds were planted (fig. 13). The fleshy layer of the seeds had less of the orange color than the usual seeds of *Z. pumila* and more of the deep red, but not so much as one usually finds in seeds of *Encephalartos villosus*. The seeds have not yet germinated, so that it is doubtful whether any F_1 generation will be obtained. It is well known that pollen of cycads is easy to germinate up to a certain age

in culture solutions. It may be that the pollen germinates in the pollen chamber and stimulates the growth of the cone and ovules, but that no fertilization takes place. One could not remove material for determining such features without danger of killing the entire cone.

All cycads, like most gymnosperms, have twelve and twenty-four chromosomes as the x and $2x$ numbers, which may account in some measure for the ease with which hybrids are obtained in this family.

So far as the names of the plants used in this study are concerned, I cannot speak with authority. For the two species of *Zamia* from Florida we have used the names *Z. pumila* and *Z. floridana*, the names used in WEBBER'S[9] studies of *Zamia* and in various papers and books from this laboratory. The *Z. latifoliolata* came from Porto Rico; the *Ceratozamia mexicana* was grown from seeds secured near Jalapa, Mexico; and the *Encephalartos villosus* came from the Botanic Gardens at Durban, South Africa, and is the form which occurs near Durban.

Records are being kept of the parents and hybrids described in this paper, so that data will be available when the time arrives for an F_2 generation.

I am indebted to P. J. SEDGWICK for the print from which fig. 1 was made, and to C. Y. CHANG for negatives and prints of figs. 2, 5, 8, 9, 10, and 13.

UNIVERSITY OF CHICAGO

[9] WEBBER, H. J., Spermatogenesis and fecundation of *Zamia*. U.S. Dept. Agric. Bur. Pl. Ind. Bull. 2. pp. 100. 1901.

CULTURE STUDIES OF PSILOCYBE COPROPHILA

KATHRYN A. GILMORE

(WITH PLATES XXXII, XXXIII)

Psilocybe coprophila was first described by BULLIARD (3) in 1791 as *Agaricus coprophilus*, and was listed under that name by both PERSOON and FRIES. It was placed in the genus *Deconica* by SMITH (12) and subsequent writers who recognize that genus. It is a widely distributed and well known form, occurring most frequently, according to REA (10), on rabbit or cow dung. Culture studies of it were reported by EIDAM (4) in 1875. The cultures used in these experiments all originated from a single basidiocarp that appeared spontaneously on rabbit dung collected in the field near Iowa City, and placed in a moist chamber. The fungus was identified as *Psilocybe coprophila* Fr., in the sense of RICKEN (11), by Professor C. H. KAUFFMAN of the University of Michigan, to whom acknowledgment is herewith extended.

The normal basidiocarp (fig. 1) has a pileus 5–16 mm. broad, at first convex, later becoming expanded and occasionally plane or even depressed. It is glabrous, somewhat hygrophanous, and subviscid when moist; the margin is rather deeply crenate, especially in age; the flesh is very thin; the color ranges from hazel to chestnut brown,[1] fading to warm buff near the margin. The gills are subdistant, unequal, adnate to subdecurrent, warm buff in color, and distinctly mottled at maturity. The stipe is 2–6 cm. in length, 1–2 mm. in the middle, tapering upward, hollow, subcartilaginous, light ochraceous buff, often floccose when young, later the lower half is white tomentose, the upper glabrous and silky-shining. The basidia are tetrasporous, $30–40 \times 10\ \mu$ (fig. 15). Flask-shaped sterile cells $20 \times 3–7\ \mu$ occur at the edges of the gills (fig. 13). The spores are distinctly purplish in mass when first shed, later blackish brown; cinnamon to snuff brown under the microscope, somewhat apiculate at both ends, and hyaline tipped, $10–12 \times 6–7\ \mu$ (fig. 14). They

[1] Names of colors are used throughout this paper in the sense of RIDGWAY.

germinate after about 24 hours in agar, in a liquid nutrient medium, or in water. The formation of a globose vesicle precedes the elongation of the germ tube (fig. 16). Such a vesicle is common in several genera of Basidiomycetes, and was described for the species under consideration by EIDAM.

Vegetative propagation is carried on by means of oidia produced on both the primary and secondary mycelia. These oidia are allantoid, vary from 4 to 10 μ in length, are approximately 1.5 μ in diameter, and are borne on special hyphae. The latter are smaller in diameter than those of normal mycelia, and are branched and coiled and tangled into globose masses 30–40 μ in diameter. They are divided into short segments by cross walls and at length break up, so that each segment becomes an oidium (fig. 8). The oidia appear in single spore cultures not long after the spore germinates. Although they are produced in greater abundance upon the primary strains, they are never lacking upon the secondary mycelia, and have not infrequently been found on hyphae bearing clamp connections (fig. 9). They do not germinate in water, but germinate in 48–60 hours in clear agar.[2] Before germination they commonly become somewhat swollen. A germ tube is first produced at one end; later, the other end usually germinates, so that the oidium is in the center of the mycelium to which it gives rise (fig. 10). The vegetative body of the fungus forms a white cottony mat. Single hyphae of the secondary mycelia normally vary from 1.5 to 2.5 μ in diameter, and when grown in a liquid medium attain a diameter of 3–4.5 μ. They branch freely and possess numerous clamp connections (fig. 11).

The fungus lends itself readily to culture studies because of its large, dark basidiospores, its ability to fruit in culture, and its short life cycle, the whole of which is usually accomplished in less than a month. Test-tube cultures have been made on bean pods, blocks of decayed oak wood, rabbit dung, cow dung, dung agar, malt agar, Cook's II agar,[3] and a modification of the latter containing less nutriment. It is known in this laboratory as IIa agar, and is made according to the following formula:

[2] 15 gm. agar in one liter distilled water; filtered.

[3] COOK, M. T., Del. Agric. Exp. Sta. Bull. 91. p. 11. 1911.

Glucose	10 gm.
Peptone	2 gm.
Dipotassium phosphate	0.25 gm.
Magnesium sulphate	0.25 gm.
Agar	15 gm.
Distilled water	1000 ml.

Basidiocarps occur most consistently on sterilized rabbit dung. Repeated attempts failed to secure their production on horse dung. IIa agar is quite favorable to normal fructification (fig. 2). An effort was made to secure an even better preparation by greater reduction of the nutrients, but without success. Abnormal basidiocarp primordia of various sorts have been produced on Cook's II and on beer agar, but they consistently failed to form true pilei or spores. Mycelial cultures were grown in liquid nutrient media but they did not fruit, even when folds of filter paper were added to provide a foundation for the basidiocarps. Mycelial cultures on both dung and IIa agar where grown in a dark locker, and in a refrigerator where the temperature was 13° C. Fruit bodies with long, slender stipes and abnormally small pilei developed in the dark locker, but mycelial growth was checked by the refrigeration, and no basidiocarps appeared until the cultures were removed to a warmer place, after which growth was renewed and normal fructification occurred. After eight months in culture, April to November, the production of fruit bodies practically ceased during December, January, and February, beginning again early in March.

Heterothallicism

Considerable investigation of the general question of heterothallicism has been carried on in recent years with species belonging to a number of genera of Basidiomycetes. Bensaude (1) gives a very complete summary of the work on sexuality in Hymenomycetes up to 1918. She worked with monosporous and polysporous cultures of *Coprinus fimetarius*, and found that a single spore gives rise to a uninucleate (haploid) mycelium; that there are two kinds of spores produced and hence two strains of haploid mycelium; that, if representatives of the two strains are grown in culture together, union will occur between a cell of one and a cell of the other; that a secondary,

diploid mycelium will be initiated as a result of this union, and she believed that fruit bodies are never formed except by a secondary mycelium. She concludes that the fusion between mycelial cells is a true sexual union without the formation of differentiated gametes, and that there is an actual alternation of generations in *Coprinus fimetarius*, the two strains of primary mycelium with a single nucleus in each cell representing the gametophyte generation, and the secondary mycelium resulting from their fusion and bearing two nuclei in each cell representing the sporophyte generation.

KNIEP (5, 6, 7) reports studies on sexuality in a number of Basidiomycetes. He agrees with BENSAUDE'S general conclusions concerning the facts on sexuality and alternation of generations in the Basidiomycetes, and is convinced that the vast majority of these forms are heterothallic. In addition he reports the production of fruit bodies by haploid mycelia in *Schizophyllum commune* and *Hygrophorus conica*, and states that in such cases the basidiocarp is haploid throughout; the nucleus of the basidium is not a fusion nucleus and divides without reduction to form the four basidiospore nuclei; the resulting spores are all alike and all produce the same strain of primary mycelium. He finds also, in *Schizophyllum commune* and *Aleurodiscus polygonius*, a situation which he believes to be common in Basidiomycetes, in which four sorts of spores instead of two are produced on each basidiocarp, giving rise to four mycelial strains. Each strain will cross with one only of the other three to produce a secondary mycelium with clamp connections. If primary mycelia from two fruit bodies of *Schizophyllum commune* that grew in different localities are crossed, however, each of the four strains from one basidiocarp will form a secondary mycelium when crossed with any strain from the other. This fact implies that the differentiation of strains depends upon a quantitative balance so delicate as to be destroyed by slight changes in environmental conditions. KNIEP also reports that in two instances primary mycelia which he had held in culture during a year's time, finally changed over into the secondary condition, suggesting again that some quantitative balance within the mycelium had been disturbed by culture conditions.

Further contributions verifying the conclusions of KNIEP and

BENSAUDE have been made by VANDENDRIES (14, 15) and MOUNCE (8, 9). A concise review of their work, as well as of the unpublished work of HANNA and NEWTON, has been given by BULLER (2). The homo or heterothallicism of a number of species has been established, but no reports have been made as yet on the genus *Psilocybe*.

The first monosporous cultures of the species under consideration were made by the poured plate method from the original basidiocarp that occurred spontaneously on rabbit dung. They constitute the F_1 gametophyte generation. Twenty-four such cultures were made, twenty-three of which produced no clamp connections and never fruited, although grown for eight months on various media in twenty-four different transfers. By the end of the eighth month, fourteen of the twenty-three cultures had died and the vigor of the remaining nine was perceptibly reduced. The latter were carried on for two more months and then destroyed. Since no clamp connections were ever found in these monosporous cultures it was concluded that the species is heterothallic, producing at least two sorts of primary mycelium, and that the union of different primary strains is necessary for the formation of a typical secondary mycelium with clamp connections.

The twenty-fourth culture formed clamp connections and fruited regularly under conditions similar to those that brought about fructification in the stock. It is possible that it was in reality not a monosporous but a polysporous culture. It is also possible, however, that it did originate from a single spore, since later a single germinating spore from the stock culture was observed to have given rise to a germ tube in which there was a clearly defined clamp connection at the first septum in close proximity to the spore, under conditions which precluded the possibility of fusion (fig. 12). Such a case was never encountered again in the great number of single spore cultures studied. No further work was carried on with the twenty-fourth culture. Oidia were produced abundantly in it, as well as in the twenty-three haploid cultures.

Numerous crosses of the twenty-three haploid cultures were made to determine the reactions between them. These crosses and the basidiocarps formed by them are referred to as representing the F_1 sporophyte generation. They were examined microscopically for

the presence of clamp connections, and then held in culture to admit of the production of fruit bodies. Not all of the possible crosses were made before some of the single spore cultures died. Enough were made, however, to establish the fact that the spores of *Psilocybe coprophila* give rise to two strains of primary mycelium (known for convenience as strains *m* and *n*), and that clamp connections occur only in those crosses in which both strains are represented. No constant difference between the two strains has ever been found, either in size, appearance, manner of growth, or any other vegetative character. Table I shows the result of the series of crosses involving F_1 gametophytes, a cross indicating the presence, and a dash the absence of clamp connections.

In those cases where members of the same strain were crossed together clamp connections were never produced, and, with one exception, clamp connections always appeared when both strains were represented in the cross. The exception was culture 12–23. Primary mycelium 12 had behaved as a normal representative of strain *m* in eight crosses, and 23 as a normal representative of strain *n* in ten crosses; hence a secondary mycelium was expected as a result of their union. Repeated examinations, however, failed to disclose clamp connections in the cross. The normal basidiocarp that it produced is the only one that has ever occurred on a mycelium without clamp connections. It seemed possible that this basidiocarp was a haploid one, such as has been described by KNIEP (5) in *Schizophyllum commune*, and has since been reported in a number of other genera, in which case it would be expected to produce spores of only one kind. In order to investigate this point, monosporous cultures representing the F_2 gametophyte generation of culture 12–23 were isolated and crossed. Contrary to expectation, clamp connections occurred in many of the crosses, and the monosporous cultures could again be divided into two strains; hence the basidiocarp produced by 12–23 must have borne spores of two kinds, and therefore must have been diploid, in spite of the fact that no clamp connections were found in it. Examination of the mycelium of this culture, made several weeks after the production of the first fruit body, revealed an abundance of clamp connections. It seems improbable that they were present and not seen in the many earlier examina-

tions. Their eventual occurrence may be accounted for by the supposition that spores shed from the basidiocarp had germinated in the culture tube and the resulting mycelia had crossed. No further peculiarities have been noted in the progeny of this cross.

Representatives of the F_2 gametophytes were crossed back with F_1 gametophytes and strains *m* and *n* identified, since one strain of

TABLE I

		Cultures of F_1 gametophytes of strain *n*											Cultures of F_1 gametophytes of strain *m*											
		1	2	4	6	8	10	11	14	15	20	23	3	5	7	9	12	13	16	17	18	19	21	22
Cultures of F_1 gametophytes of strain *m*	22	..	×	..	×	×	×	×	×	..	..	..	..	—	..	..	—	—	—	..	..	..	..	—
	21	×	..	..	..	..	..	×	..	..	..	..	..	—	..	..	..	..	..	..	..	—	—	
	19	×	×	..	..	..	..	..	×	×	..	×	..	..	..	—	..	—	—	—	..	—		
	18	..	..	..	..	..	×	×	..	..	..	..	..	..	..	..	..	..	..	..	—			
	17	×	×	..	..	..	..	×	×	..	..	..	..	—	..	..	..	—	—	—				
	16	×	×	..	×	×	×	×	×	..	..	×	..	—	..	—	—	—	—					
	13	×	×	×	..	..	×	×	×	..	..	×	..	..	—	—	..	—						
	12	..	..	..	×	×	×	×	×	..	×	—	..	..	..	..	—							
	9	×	×	..	..	×	..	..	×	..	..	×	..	..	—	—								
	7	..	..	..	..	..	..	..	×	..	..	..	..	..	—									
	5	..	..	..	..	×	..	..	..	..	..	..	—	—										
	3	..	..	..	..	..	..	..	×	..	..	..	—											
Cultures of F_1 gametophytes of strain *n*	23	—	—	..	..	—	—	—	—	..	..	—												
	20	..	..	..	..	..	—	—	..	..	—													
	15	—	..	..	..	—	..	..	..	—														
	14	—	—	—	..	..	—	—	—															
	11	..	—	..	—	..	—	—																
	10	..	..	..	..	..	—																	
	8	..	..	..	..	—																		
	6	..	..	..	—																			
	4	..	..	—																				
	2	—	—																					
	1	—																						

the F_2 gametophytes formed clamp connections when crossed with representatives of strain *m*, and the other with strain *n*.

Inheritance of abnormal basidiocarps

The great majority of the F_1 sporophytes did not fruit, and many of those which did produced abnormal fruiting bodies. These aberrancies may have been due to certain culture conditions of temperature, moisture, or nutriment; and the fact that similar abnormal forms sometimes occurred in the stock cultures of polysporous origin points to this conclusion (fig. 5). Such stock cultures always pro-

duced normal fruiting bodies on rabbit dung, and the same was true of certain of the crosses. On the other hand, some crosses, notably 11–16 and 10–22, whether on dung or on agar, consistently produced one type of abnormal fruit body having a normal stipe but not a normal pileus. Instead, the apex of the stipe is swollen and hollow, and from the resulting clublike structure there grow out in all directions thin, irregular folds of tissue that function as gills, bearing a hymenium producing viable spores (fig. 3). It seemed possible that the tendency to produce this particular abnormal form might be inherent in the mycelium and transmitted from generation to generation. ZATTLER (16) describes such a case in *Schizophyllum commune*, where certain strains carried in their inheritance the tendency to produce "knäuel" fruit bodies, and transmitted it as a simple Mendelian recessive to their offspring. In order to investigate this point, single spore cultures were isolated by the poured plate method from basidiocarps of 11–16 and 10–22.

In the case of 11–16, thirteen monosporous F_2 gametophyte cultures were isolated, five of which were found to belong to one strain and eight to the other. Five crosses that produced clamp connections were held in culture. They have not been very prolific, having produced only six basidiocarps, all on rabbit dung. Of these, one was normal and five were abnormal. None of the abnormal ones was typical of the type described as occurring on the parent stock.

More convincing results were secured with the offspring of cross 10–22. Eight monosporous cultures of the F_2 gametophyte generation were isolated and crossed in every possible combination. Again the presence or absence of clamp connections in the crosses served to divide the monosporous cultures into two strains, and by crossing representatives of each strain with the gametophytes of previous experiments it was possible to identify strains *m* and *n*. Cross 12–23, already described, was used as a check. All of the crosses involving F_2 gametophytes from 10–22 (abnormal) and 12–23 (normal) were made in December 1924, and held in culture on rabbit dung during the same period of time and under the same conditions. The record of basidiocarp formation in the two cases affords an excellent opportunity for comparison of the fructification of the offspring from a cross producing a preponderance of normal with one

producing a preponderance of abnormal basidiocarps. Sixteen crosses were made that involved F_2 gametophytes from the normal culture 12–23, forming fifteen basidiocarps. Thirteen of these were entirely normal. Two, both produced as the result of a single cross, were merely convolute masses of hymenial tissue without a stipe, described later as the third type produced by the abnormal strain. Seventy-three crosses were made involving F_2 gametophytes from the abnormal culture 10–22, producing ninety-six basidiocarps. These could be divided into four types, not sharply distinguished, but grading into one another. The first type is the normal basidiocarp (fig. 1). Thirty-seven of these were produced, sixteen of which occurred in crosses between F_2 abnormal gametophytes and F_1 or F_2 normal gametophytes. The second type has a stipe and an abnormal pileus. Representatives of this type vary widely in the shape of the pileus and in the distribution of the hymenium-bearing tissue (fig. 7); many of them approach the typical abnormal form already described. Seventeen basidiocarps of this type were produced. The third type has neither stipe nor pileus; instead, a mass of basidiocarp tissue of no regular shape is formed. It may grow horizontally over the substratum, or it may become erect; but at its apex it always produces more or less hymenium-bearing tissue in folds and convolutions of various sorts. Nineteen basidiocarps of this type were produced. These three types all produce viable spores. The fourth type is simply a mass of basidiocarp tissue and produces no spores (fig. 6). Twenty-three such abortive bodies were produced; hence the crosses involving F_2 gametophytes of 10–22 (abnormal) produced normal and abnormal basidiocarps in the ratio of 1–1.6, while the crosses involving F_2 gametophytes of 12–23 (normal) produced normal and abnormal basidiocarps in the ratio of 6.5:1. Since the two series fruited during the same interval of time and under the same conditions, it is obvious that the capacity to form normal or abnormal fruit bodies is affected by inheritance. It cannot, however, be expressed in a simple Mendelian ratio. Many F_2 sporophytes from the abnormal cross produced both normal and abnormal basidiocarps from the same mycelium, a circumstance that would not occur if the capacity for forming abnormal basidiocarps were either strictly dominant or strictly recessive to the capacity for forming

normal ones. It is possible that the tendency to fruit abnormally is present in the mycelium of these crosses, but is able to express itself only under certain environmental conditions. The amount of moisture present is possibly the critical factor, since it has been the most variable one and the hardest to control.

It seemed desirable to make some study of the F_3 generation of an abnormal strain, and accordingly single spore cultures were isolated from a basidiocarp formed by sporophyte 46–48. Cultures 46 and 48 were F_2 gametophytes from the abnormal strain 10–22, and the secondary mycelium produced by crossing them had formed both normal and abnormal basidiocarps. Single spore cultures isolated from one of the normal basidiocarps were crossed with each other and with F_2 gametophytes from the normal cross 12–23. These crosses produced nine basidiocarps, two abnormal and seven normal. Fig. 4 shows a normal and an abnormal basidiocarp produced simultaneously in one of these cultures.

Oidial studies

The oidia of this species were described by EIDAM (4). Working long before the sexuality of the Basidiomycetes was understood, he believed the oidia to be male cells or spermatia. He looked in vain for the female organs, and concluded that they appeared less frequently than the spermatia, which were common and abundant so as to insure fertilization. Oidia similar in appearance and in manner of formation have been found repeatedly on the primary mycelia of other Hymenomycetes. VANDENDRIES (14) reported their occurrence on *Hypholoma fasciculare*, and THOM and LATHROP (13) found them on the secondary mycelium of a form which they isolated from fermenting bagasse and believe to be *Psilocybe atomatoides* Pk.

It seemed worth while to compare the reactions of oidia occurring upon the primary and secondary mycelium of *Psilocybe coprophila*. The poured plate method was used to isolate cultures arising from single oidia. The oidia are hyaline, and so small that it is difficult to be sure that a given field in the agar plate contains but one; consequently the possibility of error is greater in all of the oidial experiments than in those depending upon the isolation of the large, dark basidiospores.

Six single oidial cultures were isolated from F_1 gametophyte 13 of strain *m*, and six from F_1 gametophyte 14 of strain *n*. Clamp connections were not found in any of these cultures. In crosses they behaved exactly like the parent cultures from which they were derived; hence it was concluded that the oidia from a haploid mycelium of either strain are haploid, as would be expected, and give rise to haploid mycelia of the same strain.

Eleven single oidial cultures were isolated from the stock culture of polysporous origin. Each of them produced a diploid mycelium

TABLE II

	02*	05	07	01	03	04	06
54	×	×	×	—	—	—	—
26	×	×	—	×	×	×	×
06	—	—	×	—	....	....	—
04	....	....	....	....	....	—	
03	....	....	....	....	—		
01	—	—	×	—			
07	×	×	—				
05	—	—					
02	—						

* Cultures 02 and 05 are single oidial cultures from the F_1 sporophyte 14–19, which formed a secondary mycelium when crossed with representatives of either strains *m* or *n*.

Culture 07 is a single oidial culture of strain *n* from the F_2 sporophyte 48–46.

Cultures 01, 03, 04, 06 are single oidial cultures of strain *m* from the F_2 sporophyte 14–19.

Culture 26 is an F_2 gametophyte of strain *n* produced by F_1 sporophyte 12–23.

Culture 54 is an F_2 gametophyte of strain *m* produced by F_1 sporophyte 12–23.

with clamp connections. Two weeks later two additional cultures of the same sort were isolated from another stock culture of polysporous origin, and these also were diploid.

Six single oidial cultures were isolated from the F_1 sporophyte 14–19 and one from the F_2 sporophyte 45–48. Unlike the oidial cultures from stock, these seven were all haploid. Crosses involving them were made, and are recorded in table II. Culture 54 is a representative of strain *m*, and 26 of strain *n*. No explanation is offered for the fact that cultures 02 and 05 formed clamp connections when crossed with either 54 or 26; nor for the fact that, having crossed with both 54 and 26, they refused to cross with each other, or with cultures

01 and 06. Bacterial contamination that was present in this series of crosses may have interfered with normal reactions. The five cultures other than 02 and 05 reacted as normal haploid mycelia; 01, 03, 04, and 06 representing strain *m*, and 07 representing strain *n*.

The suggestion presented itself that the oidia which gave rise to these cultures were perhaps not borne on the secondary mycelia of the crosses 14–19 and 45–48 from which they were taken, but on the primary mycelia 14, 19, 45, and 48, which might still be maintained in the culture tubes. In order to investigate this possibility, transfers of mycelium from cross 14–19 were made to agar plates. While the resulting cultures were still young, the tips of radiating hyphae with clamp connections, but not bearing oidia, were cut off and transferred again. Thus plate cultures were prepared which had their origin from secondary mycelium only, and could not be mixed cultures bearing both diploid and haploid hyphae and oidia. This experiment was repeated several times, and in every case, after a few days, oidia appeared abundantly in the culture. An attempt was made to isolate cultures from single oidia produced by such cultures, but only two of them lived. These two were both haploid.

Eight single oidial cultures were isolated from the F_2 sporophyte 52–53. Seven of them were haploid and the eighth diploid. Since the oidia from stock and those from known crosses had behaved differently, in spite of the fact that both came from diploid mycelia, it seemed wise to check the results obtained from the stock oidial cultures. Four oidia from stock were isolated six weeks after the first ones had been secured. This time all four produced not diploid, but haploid mycelia without clamp connections.

The behavior of oidia from diploid cultures has been so variable that interpretation is difficult. Seventeen single oidial cultures have been isolated from diploid mycelia formed by crossing two primary strains. Oidia from three such crosses were used. Of these, sixteen were haploid. Two of these haploid cultures originated from oidia that were produced on plates where only a diploid mycelium was growing, so they could not have been formed by the original haploid strains that united to produce the diploid mycelium; hence diploid mycelia must give rise, at least in some cases, to haploid oidia. In order that this may take place there must be a segregation of nuclei

within the secondary mycelia. Either portions of growing secondary mycelia are continually breaking down into the primary condition and these portions produce the oidia, or segregation of nuclei occurs in diploid mycelia at the time of the formation of oidia, making the oidia haploid while the mycelia that bear them remain diploid.

The seventeenth oidial culture from a known cross was diploid. It might have originated from a small bit of diploid mycelium or from two unlike haploid oidia erroneously taken for a single germinating oidium. On the other hand, the belief that it originated from a single diploid oidium is supported by the fact that so many diploid cultures were secured from oidia produced by stock cultures of polysporous origin. In the latter case, the first thirteen cultures were all diploid, and it seems extremely unlikely that so many errors were made in isolating cultures; hence, diploid mycelia must sometimes give rise to diploid oidia. Later oidial cultures from an older stock culture were all haploid. This implies again that segregation of nuclei had taken place. But since the ratio of diploid to haploid cultures from stock oidia is 13:4, and only 1:16 in the case of oidial cultures from a known cross, and since the stock cultures from which the thirteen diploid oidial cultures were isolated were younger than those from which the four haploid oidial cultures were isolated, it is evident that segregation must take place less readily in cultures of polysporous origin.

Summary

1. *Psilocybe coprophila* Fr. has been found to be a heterothallic species producing two strains of primary mycelium, the union of which is necessary for the production of a secondary mycelium with clamp connections, and of basidiocarps.

2. The capacity for the formation of abnormal basidiocarps is greater in some crosses than in others, and is transmitted from generation to generation, although not as a simple Mendelian character.

3. Oidia are produced on both primary and secondary mycelia. Those produced by primary mycelia germinate to form haploid mycelia of the same strain as the parent culture. Those produced by secondary mycelia sometimes germinate to form diploid mycelia. At other times they germinate to form haploid mycelia, segregation

of nuclei occurring before the oidia are produced. This segregation seems to take place less readily in cultures of polysporous origin than in a diploid mycelium formed by crossing two primary strains.

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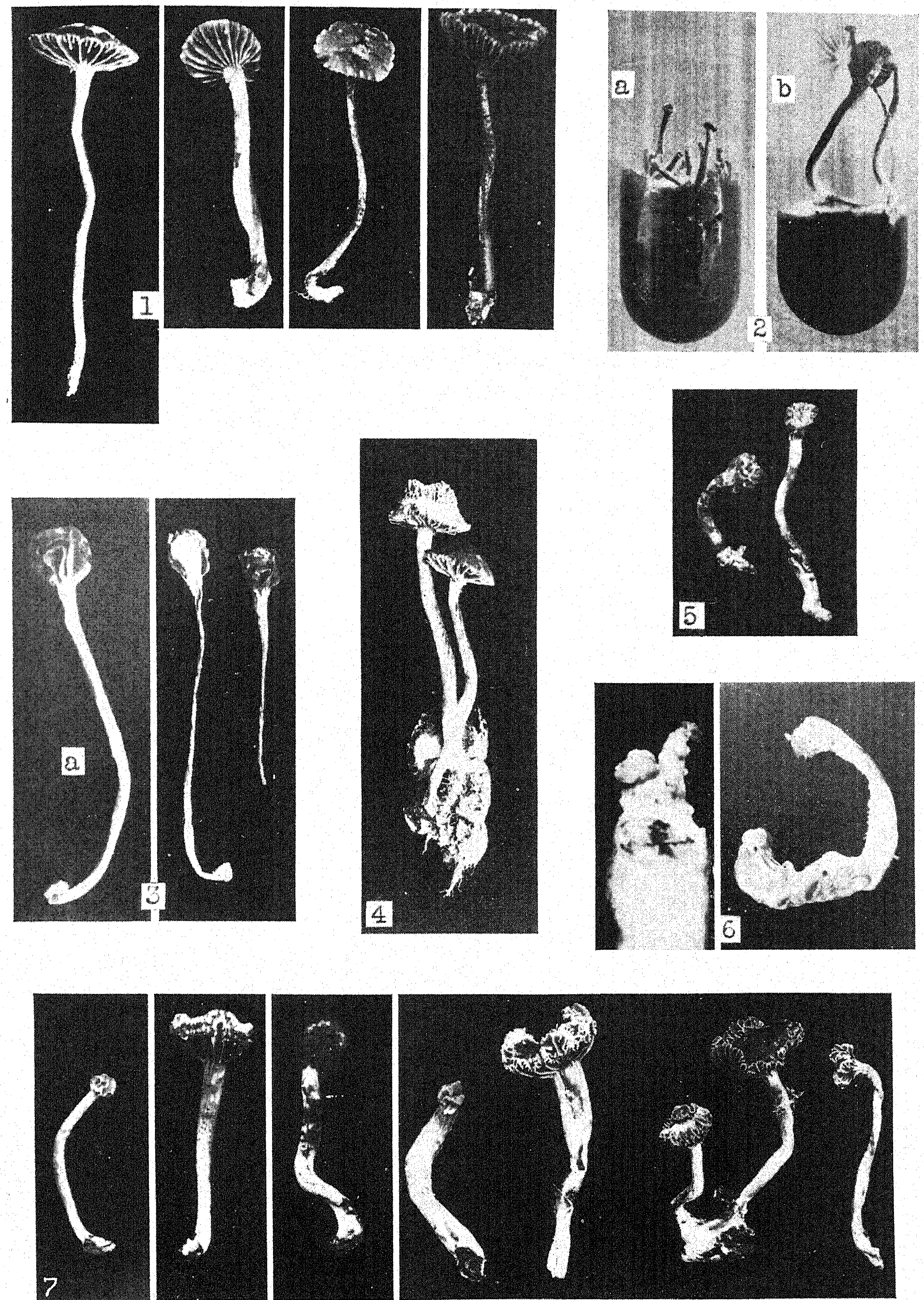
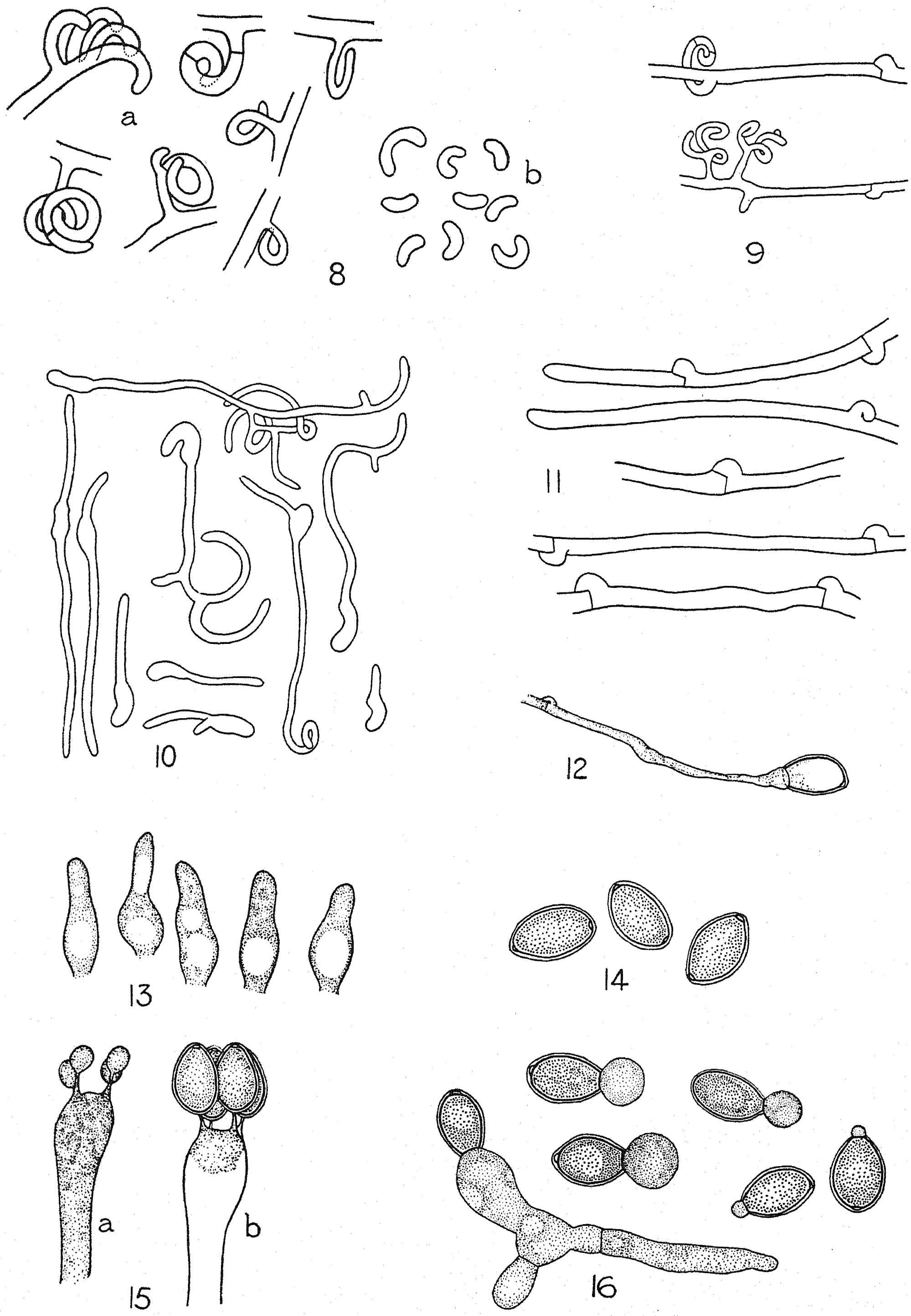


GILMORE on PSILOCYBE



GILMORE on PSILOCYBE

EXPLANATION OF PLATES XXXII, XXXIII

PLATE XXXII

FIGS. 1–7 photographed natural size; reduced one-eighth in reproduction.

FIG. 1.—Normal basidiocarps of *Psilocybe coprophila*.

FIG. 2.—Normal fructification of stock culture on IIa agar: *a*, primordia; *b*, mature basidiocarp.

FIG. 3.—*a*, Abnormal basidiocarp of type produced by cultures 11–16 and 10–22: *b*, same basidiocarp cut longitudinally.

FIG. 4.—Normal and abnormal basidiocarps produced simultaneously by F_3 sporophyte culture on rabbit dung.

FIG. 5.—Abnormal basidiocarps produced by stock culture on IIa agar.

FIG. 6.—Masses of undifferentiated basidiocarp tissue.

FIG. 7.—Various types of abnormal basidiocarps with stipes and pilei.

PLATE XXXIII

FIGS. 8–16 drawn with camera lucida; figs. 8, 11, 13–16 $\times$ 1920; figs. 9, 10, 12 $\times$ 1515; reduced one-half by reproduction.

FIG. 8.—*a*, oidiophores; *b*, oidia.

FIG. 9.—Oidiophores occurring in close proximity to clamp connections.

FIG. 10.—Germinating oidia.

FIG. 11.—Clamp connections from stock cultures.

FIG. 12.—Germinating spore with clamp connection at first septum of germ tube.

FIG. 13.—Flask-shaped sterile cells occurring at gill edges.

FIG. 14.—Basidiospores.

FIG. 15.—Basidia: *a*, young; *b*, mature.

FIG. 16.—Germinating basidiospores.

OSMOTIC PRESSURE OF CELL SAP AND ITS POSSIBLE RELATION TO WINTER KILLING AND LEAF FALL

FLOYD W. GAIL

(WITH THREE FIGURES)

Introduction

The fact that most coniferous trees and some of the angiosperms do not shed their leaves every year has attracted considerable attention among scientists interested in plant life. The large number of evergreen plants in Idaho, Oregon, and Washington was instrumental in causing the writer to begin this investigation. Numerous studies have been made on the density and sugar content of the cell sap, but, so far as the writer has been able to ascertain, no studies have been made at regular times throughout an extended period. The work here reported covered nearly three years.

SCHULTZ (15) studied reserve materials in evergreen leaves in winter. All gymnosperms studied were found to be free from starch except *Gnetum gnemon*, in which the green cells contained some starch granules. Among the angiosperms, all monocotyledons and some dicotyledons were free from starch. A few contained more or less starch in the leaves. According to the investigations of MER (10), LIDFORSS (9), and others, almost all of the evergreen plants of northern and middle Europe lose the starch from the green cells of the leaves during winter. They have also shown that a close relation exists between the sugar content and frost hardiness. MIYAKE (11) found that, generally speaking, starch in evergreen leaves began to decrease in November, reaching its minimum during January and the beginning of February, and increased again from the end of February. Many evergreen leaves in Tokyo and other parts of middle and southern Japan contain more or less starch, while it is entirely absent in some species in the coldest time of winter. The majority of evergreen leaves in the northern part of Japan lose nearly all of their starch from the mesophyll and guard cells in winter.

WARMING (17) considers that the presence of oils and fats in leaves in the winter may enable the plant to withstand low temperatures, in that fatty oil in the form of an emulsoid prevents the subcooling of the plant tissue and increases the power of resistance to frost. TUTTLE (16) worked on the reserve material in *Linnaea*, *Pyrola*, and *Picea*, and found that most evergreen plants are destarched in northwestern Canada as early as October, and then contain a large amount of oil. Exposure to high temperatures in the case of *Linnaea* induces the formation of starch in darkness. The starch disappears again when exposed to moderately low temperature for about eight days, but the leaves are killed if exposed to extremely low temperatures when filled with starch. LEWIS and TUTTLE (8) have shown that the maximum osmotic pressure is reached in *Picea* and *Linnaea* toward the end of March. *Pyrola* shows a fairly steady decrease from the middle of December until June. The variation of the sugar content closely follows the variation of the osmotic pressure. The sugars show a decided concentration during the winter months. In the living leaves of *Pyrola*, ice formation does not begin until a temperature of $-31.6°$ C. is attained.

HARVEY (4) found that the relative injury to varieties of cabbage and lettuce at low temperatures is dependent upon varietal differences in the ability of these plants to harden. Cabbage which had been placed in dark chambers and exposed for five days to $3°$ C. was not injured by thirty minutes, exposure to $-3°$ C., although frozen stiff. He also found the average excess of the freezing point depression in the cell sap of hardened cabbage plants over the cell sap of non-hardened to be nearly $0.1°$. He considered this slight difference insufficient to account for the resistance to low temperature shown by hardened plants which are not injured by being frozen at a temperature $3°$ below the killing temperature for non-hardened plants.

MÜLLER-THURGAU (12) in 1880 gave the freezing point of potatoes as $-1.8°$ C. WRIGHT and HARVEY (18) report that the freezing point of potatoes tends to rise as the season advances. HARVEY and WRIGHT (6) state that, during the usual weather conditions which precede the first killing frost in autumn, the night temperature is usually somewhat above $32°$ F., but low enough to increase

the accumulation of osmotically active sugar in tomatoes with a consequent lowering of the freezing point of the plant sap. WRIGHT and TAYLOR (19) state in their summary that potatoes freeze more quickly when exposed to rapidly diminishing temperature than when it falls slowly.

KORSTIAN (7) found that the density of the cell sap changes during the day, having a minimum in the morning, increasing as the day advances, and decreasing toward evening. Newly developed leaves have uniformly lower sap densities in July than one-year-old leaves. In September the relative densities were found to be variable; about one-half of the species showed higher density in the leaves of the current year, and the other half in those of the second year. By December and January, however, all of the species tested showed uniformly higher densities in the second year leaves. Starch was not detected in any of the conifers or evergreen shrubs in either January or February. Osmotic pressure in plants is more rapidly changed by fluctuation in moisture conditions of the site than by temperature or light.

Method

It is well known that the osmotic pressure is proportional to the freezing point depression. In this investigation, the freezing point depression was determined by the use of the Beckman thermometer, and the osmotic pressure was computed on the basis of the freezing point depression.

The needles from the current year's growth only were picked from the trees. They were then at once ground through a meat grinder, using the fine knife. In most cases this pulp was frozen and then the sap pressed out, using a definite amount of pressure and a definite amount of pulp. Little difference in the freezing point depression resulted from freezing the pulp. The fact that there was so little difference in the freezing point depression produced by freezing the pulp probably may be explained by the fact that the cells of the leaves were so largely broken by being ground into the pulp. The freezing point depression was then determined. Tests were made at least once a month for a period of nearly three years.

The non-deciduous trees used retained their needles four or more years. The non-deciduous trees used were *Pinus ponderosa*,

Pseudotsuga taxifolia, and *Abies grandis*. The non-deciduous, broad leaved shrubs used were *Ceanothus velutinus* and *Pachistima myrsinites*.

Discussion

Non-deciduous trees and shrubs

The cell sap was tested each month throughout the years 1922 to 1924 inclusive. Fig. 1 shows that the osmotic pressure of the cell sap in every species studied increased very rapidly during December

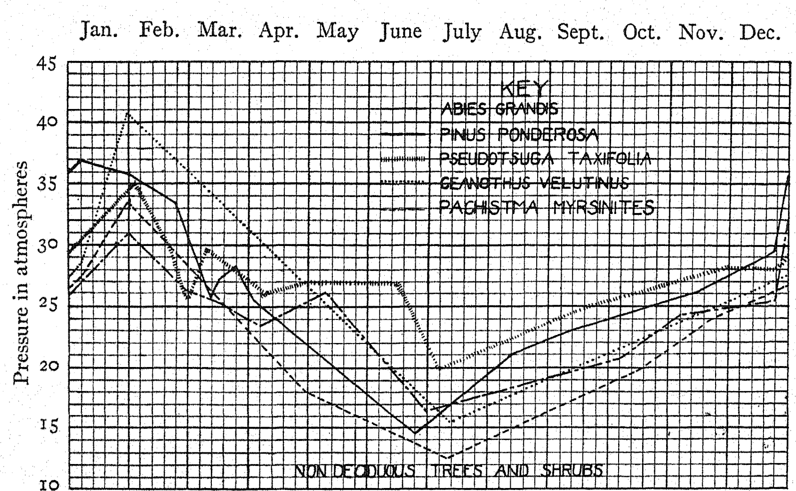


Fig. 1

and January, and that the highest osmotic pressure was during the last week of January or the first week of February. The osmotic pressure then decreased until the last week of June or the first two weeks of July. This indicates that the growth in this region ceases largely by the middle of July. Photosynthesis, however, continues but the products are stored. This causes a gradual increase in osmotic pressure in all species during August, September, October, and November (fig. 2).

Lewis and Tuttle likewise found that the osmotic pressure of the cell sap of *Pyrola* showed a fairly steady decrease from the middle of December until June. Korstian also reports a similar fact, but attributes the increased osmotic pressure to the dry season.

This he considers reduces the amount of available water, and as a result the osmotic pressure is increased. In this region the dry season begins about July, continuing for about three months. During this time the osmotic pressure does commence to increase gradually. The rainy season usually begins in October or November, however, but the osmotic pressure still continues to increase. This leads the writer to believe that there is some other factor than the reduced amount of available water which causes increased osmotic pressure.

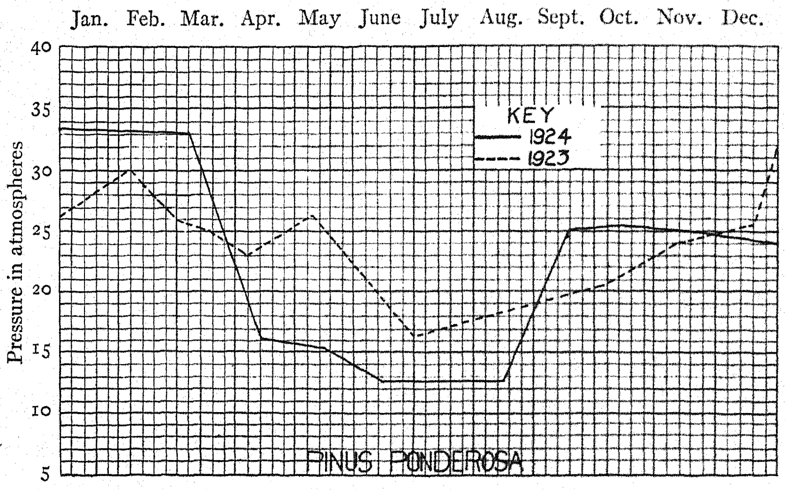


FIG. 2

Perhaps the gradually increasing osmotic pressure during August, September, and October is due largely to the cessation of growth and accumulation of carbohydrates not used in growth. The growth may have been inhibited by the dry season. The continued increased osmotic pressure of the cell sap during November and December, when there was an abundance of water in the soil but a lower temperature and little or no growth, the writer attributed to the lower temperature. The enzymes at this time are changing some of the starches to sugars, oils, etc., which increases the osmotic pressure.

The highest osmotic pressure recorded was 40.08 atmospheres in *Ceanothus velutinus*. This was during the last week of January.

The lowest osmotic pressure was recorded as 10.51, during the second week of July. This was in the cell sap of *Pachistima myrsinites*. Repeated readings demonstrated that, as the altitude increased and the temperature was lower, there was increased osmotic pressure in the same species. The fluctuations in the curves (fig. 1) showing the osmotic pressures during February and March are the results of cloudy days alternating with days of sunshine. Continuous cloudy weather caused a lowering of the osmotic pressure, since the amount of carbohydrates produced by photosynthesis is reduced, and at the same time growth is probably taking place.

The osmotic pressures shown in fig. 1 are the averages for the two years 1922 and 1923. The investigation was continued, however, to March 1925. Fig. 2 shows the average osmotic pressure for *Pinus ponderosa* for the two years (taken from fig. 1), and also for the year 1924. The average osmotic pressures of the cell sap for the two years is quite comparable with the osmotic pressure of the cell sap for the year 1924 until the month of December. The average osmotic pressure for the years 1922 and 1923 increased rapidly during the last two weeks of December, while for the year 1924 it did not increase during December or through the remaining winter months. Table I shows the temperatures for the month of December for the years 1923 and 1924, as recorded by the United States Department of Agriculture, Weather Bureau of Latah County of the State of Idaho. It will be seen that, in December 1923, the temperature varied little until the thirtieth, when the temperature was 20° F. and on the thirty-first it was −2°. This was followed by four days in January of zero weather or below, but the lowest temperature was −7°. The greatest drop in a single day was 22°. This was followed by temperatures considerably above zero. The highest temperature during the entire month was 45°. During 1924 the highest temperature for the same month was 56°, and it will be seen (table I) that in twenty-four hours there was a drop of 60°. This was followed by nine days when the temperature went as low as −17° on two days, and at no time was it above zero. The temperatures in the Selway National Forest were similar to those for Latah County, but the extremes were even more severe. A drop of 67° took place in less than forty-eight hours. This was followed by eleven days when the

temperature was −19° for two days and below zero every day except one, when the temperature was 0° F.

It is an interesting fact that, during the years 1922 and 1923, the needles of the *Pinus ponderosa* forests did not winter kill. During the winter months of 1924–1925, however, they were completely winter killed in large areas. This was true in the Spokane region, in the Nez Perce prairies, to a considerable extent in Latah County, and in other regions of northern Idaho, eastern Washington, and

TABLE I

TEMPERATURES (°F)

YEAR	DECEMBER																		
	1	7	14	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1923......	28	36	36	45	45	45	36	37	36	34	26	35	30	33	31	43	31	20	− 2
1924......	45	30	56	− 4	−17	−17	−10	− 2	0	−14	−12	− 4	4	9	17	21	38	39	39

parts of Montana. Most of the trees were not killed, since they developed new needles from the buds. Since the killing of the needles did not occur in either 1922 or 1923, when there was no such sudden drop of temperature, the writer is inclined to attribute it to the sudden lowering of the temperature which occurred in December 1924.

LEWIS and TUTTLE found that the variation of the sugar content closely follows the variation of osmotic pressure. The sugars show a decided concentration in winter in the case of *Pyrola*.

PEARSON (13) states that when the soil temperature falls to 32° F. or even a few degrees above 32°, the soil moisture ceases to be available. If this condition persists continually over a long period during which transpiration is favored by sunshine and wind, the result may be fatal to a tree which is unable to endure extreme desiccation. The temperature up to the time of the sudden drop was unusually high, being 40° or higher most of the time.

The ground at the time of the sudden drop in temperature was practically destitute of snow. PEARSON states that, during the snowless period, the soil temperatures taken in the open down to six inches in depth are usually several degrees higher than the mean or even the maximum air temperatures. Since the period of low tempera-

tures was nine days and the temperature of the air preceding this time was unusually high, the temperature of the soil, for any considerable length of time, could not have been below the point (32° F.) at which the soil moisture ceases to be available.

During the winter of 1917, GAIL (2) found that there was winter killing of the needles of *Pinus ponderosa*. The wind blew continuously and very rapidly from the southwest for a considerable period of time. The following spring the needles were found to be brown and killed on the exposed side of the trees, while on the protected side they were alive and green. The average velocity of the wind per hour during the cold spells of January 1924 and of December 1924 were respectively 11.4 and 11.53 miles. The needles of the yellow pine were not killed in any of the regions mentioned during the cold spell of January 1924, but they were killed the next winter during the cold spell of December 1924. The difference in the velocities of the wind was only 0.13 of a mile per hour. The cold spell of December 1924 did continue approximately four days longer than the cold spell of January 1924. These two differences, being so little, can hardly explain the winter killing of the needles of the later date. The wind velocity was not excessive in either January or December of 1924. The prevailing wind was from the southwest, but all of the needles on most of the trees were killed in December 1924. Had this been due to wind, the leaves on the southwest side only would have been killed, since the prevailing winds were from that direction. The writer does not consider the winter killing of the needles was due to desiccation brought about by either the low temperature of the soil or evaporation due to the wind. The low temperature in itself was not the cause, but rather the sudden fall of the temperature. This was so sudden that the starches were not changed to sugars, oils, etc., which resulted in the osmotic pressure of the cell sap not being increased, as fig. 2 shows, and consequently the protoplasm of the cells was frozen. This the writer considers resulted in the winter killing of the needles. This seems to be in accord with the work of TUTTLE, who found the leaves of *Linnaea* were killed if exposed to extremely low temperature when filled with starch. It also agrees with the work of WRIGHT and TAYLOR on potato freezing.

DECIDUOUS TREES AND SHRUBS

The trees used were *Larix occidentalis* and *Fraxinus lanceolata*. Tests to show the osmotic pressure of the cell sap were begun as soon as the leaves were sufficiently well developed, which was about the middle of May. Tests were continued once each month or more until the falling of the leaves in early November. Fig. 3 shows the osmotic pressure in May to be about 15 atmospheres for both trees.

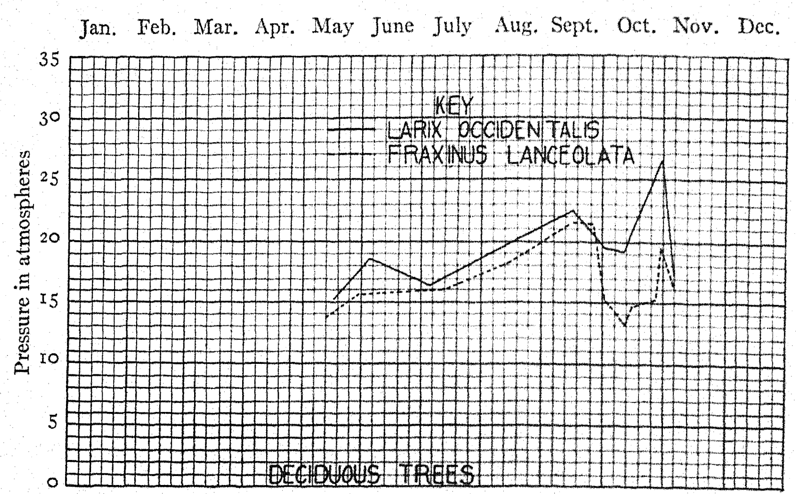


FIG. 3

This increased for both trees slightly until about the middle of September, when there was a decrease. About the middle of October the osmotic pressure increased again rather rapidly. It increased to 26.53 atmospheres in *Larix occidentalis* and to about 19.5 atmospheres in *Fraxinus lanceolata*. The temperature became lower the last week of October; heavy frost occurred during the first week of November, and the leaves were shed November 5. The osmotic pressure of the leaves at this time (immediately after being shed) had dropped to 8.43 atmospheres in *Larix*, and to 5.4 atmospheres in *Fraxinus*. This would seem to indicate that there had been a transfer of the sugars from the leaves to the trunk, and that as a result the osmotic pressure decreased. Tests were made on alternate days during the last week of October and the first week of November, in

order to determine as accurately as possible the changes that were taking place.

Conclusions

1. The lowest osmotic pressure in the cell sap of the non-deciduous plants studied is during the last half of June and the first half of July.

2. There is a gradual increase in the osmotic pressure of cell sap in the non-deciduous plants studied, throughout the months of August, September, October, and November. The stored starches are changed to sugars, oils, etc., which increase the osmotic pressure. The change of starches to sugars and oils is brought about by enzymes as the temperature lowers.

3. Growth of the conifers under consideration practically ceases by the middle of July. The drought during July, August, and September may inhibit growth. Photosynthesis continues, however, and the products are stored, resulting in increased osmotic pressure.

4. There is usually, in the non-deciduous plants studied, a sudden increase in the osmotic pressure of the cell sap during December and January. If the temperature drops low enough too suddenly, however, the osmotic pressure does not increase. This the writer considers results in the freezing of the protoplasm, and, if the freezing is severe enough, kills the leaves, causing them to turn brown and fall. It may even kill the entire plant.

5. That the osmotic pressure does not increase is probably explained by considering that the sudden drop in temperature does not give sufficient time for the enzymes to change the starches to sugars, oils, etc., before freezing takes place. This perhaps explains the winter killing of the needles of *Pinus ponderosa* which took place in December 1924.

6. Reduced amount of light, due to cloudy weather, in both non-deciduous and deciduous plants studied, causes a decrease in the osmotic pressure of the cell sap.

7. There is a consistent increase in the osmotic pressure of the cell sap of the non-deciduous trees and shrubs studied as the temperature becomes lower during the fall and winter months.

8. There is no consistent increase in the osmotic pressure of the

cell sap of the deciduous trees studied, as the temperature becomes lower in September, October, and November.

9. The osmotic pressure increases with the same species of non-deciduous plants studied, growing in different altitudes as the altitude increases.

Acknowledgments are due to Mr. E. M. KEYSER, assistant meteorologist, United States Weather Bureau, Spokane, Washington, for wind data; to Mr. M. L. MARSH of the United States Forest Service, Kooskia, Idaho, for temperatures in the Selway National Forest; and to Mr. H. A. BRUENN, Winchester, Idaho, for other temperature data.

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STRUCTURE AND CULTURAL HISTORY OF A MYCETOZOAN FOUND IN TOBACCO PLANTS WITH MOSAIC-LIKE SYMPTOMS[1]

(WITH PLATES XXXIV–XXXVII AND TWO FIGURES)

PHILIP M. JONES

Introduction

The purpose of this paper is to give a description and the life history of a mycetozoan obtained from tobacco plants affected with mosaic-like symptoms. The organism is provisionally placed in the genus *Plasmodiophora*, because it is nearer the structure of this genus than any other which has been described. It should be noted, however, that its method of depositing spores is different from any *Plasmodiophora* hitherto described. As the species is probably new it is named **P. tabaci,** n. sp.[2]

Methods

To demonstrate the presence of the organism in tobacco tissues the following method is used. The tissue is killed with osmic acid vapor and then fixed in Schaudinn's fluid without acetic acid for one hour. Following this, the tissues are dehydrated and imbedded in paraffin, sectioned, and stained in Haidenhain's haematoxylin (long method). Even better results are obtained when the tissues are placed in Knop's solution for three days before fixation.

The organism is cultured from tobacco tissues in a dilute Knop's solution. One gram each of KNO_3, KH_2PO_4, Mg_2SO_4, and $Ca(NO_3)_2$, and a trace of ferric phosphate are dissolved in 200 cc. of distilled water. This solution is placed in Petri dishes or flasks with cover glasses, and then sterilized at 15 pounds for one half-hour. The plant tissues are cut into small pieces, washed in 1–1000 mercuric chloride solution, rinsed repeatedly with sterile water, and then placed in the

[1] This paper is being published on its recent receipt, at the expense of the author.

[2] JONES, P. M., A mycetozoan found in tobacco plants with mosaic-like symptoms. Abst. in Phytopath. 16:67. 1926.

Knop's solution and allowed to incubate 7–15 days. By this method either amoebae or flagellates or both are obtained. By repeated transfers the organism was maintained in culture for over two years. To study the organism the cover glasses are removed, wiped on the under side, and examined under the microscope.

For cytological studies the organism is killed by exposure to osmic acid fumes for one-fourth minute, and then placed in Schaudinn's solution (95 per cent alcohol saturated with mercuric chloride). The fixed material is stained in Haidenhain's iron haematoxylin (short method), and mounted in balsam.

Occurrence in tissues of tobacco plants

By the method just given, plasmodia have been demonstrated in the tissues of the tobacco plant. The organism is intracellular, flowing from cell to cell by its pseudopodia (figs. 1, 3, 4, 59). It may lie close to the cell wall, or around the nucleus or the chloroplasts. Little dots frequently are seen on the periphery of the nuclei and of the chloroplastids. These dots seem to be similar to the inclusions found in the cysts which the organism forms when cultured in Knop's solution. Often the nucleus of an infected cell is found in division. Disintegration of chloroplasts apparently is the first visible response on the part of the cell to the presence of the organism. Later the cell walls disintegrate and disappear (fig. 4).

Life cycle in culture

By changing the food, temperature, and moisture conditions of the culture medium, the various stages of the life cycle have been determined. The amoebae and flagellates which develop when the tobacco tissues are placed in Knop's solution become encysted under proper conditions. Each of these cysts later develops into an amoeboid organism. A number of these may fuse to form a plasmodium, which in turn gives rise to free spores, in a way characteristic of this species. These free spores, after encysting, give rise to amoebae of the "limax" type.

In the cyst stage of the life cycle of the mycetozoan in culture there occur inclusions which may be parasites of the *Plasmodiophora*. So far, the plasmodium, with or without bodies which appear similar

to the inclusions found in the cysts in culture, is the only stage which has been found in killed and stained tobacco tissues. Amoebae and flagellates which differ somewhat in shape and size from those

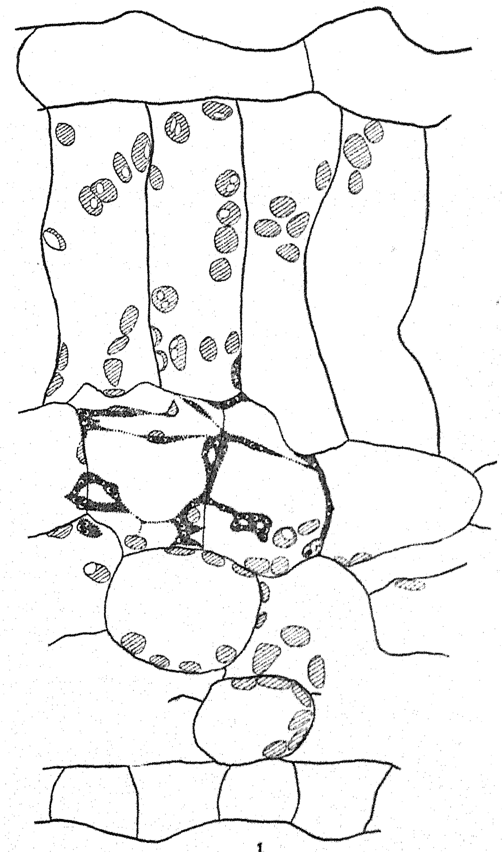

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FIG. 1.—Semi-diagrammatic camera lucida sketch of section through leaf of tobacco with mosaic-like symptoms, showing plasmodium in mesophyll; ×760.

obtained from tobacco tissues, have been cultured in Knop's solution from tomato plants affected with mosaic, from potato plants affected with leaf roll, and from the intestines of aphids feeding on tobacco and tomato plants with mosaic-like symptoms and on potato plants showing the symptoms of leaf roll (figs. 71–75).

Amoeboid stage

Morphology of "limax" type.—The endoplasm is filled with food vacuoles which contain bacteria in process of digestion. These bacteria probably are carried into the culture by the plant tissues. Some amoebae were found to be full of food vacuoles, especially at the posterior end; some had none; and in the majority of cases there were a few only (figs. 5, 6, 7). The contractile vacuole is variable, being present in some cases and absent in others. When present it is always posterior to the nucleus.

The resting nucleus is spherical in shape with a distinct nuclear membrane (fig. 12). Between this and the small karyosome lies a clear area. The karyosome is centrally located and appears as a large compact sphere which sometimes stains homogeneously; in other cases the central portion stains much less darkly than the periphery, and a small deeply staining granule, which may be a centriole, can be seen in the center.

Movement.—The body of the amoeba has one constant character, namely, it progresses by means of one blunt broadly rounded or lobose anterior pseudopodium (figs. 12, 68). While the general direction of movement is in a straight line, there is not a constant flowing movement, but rather an alternation of pseudopodium formation from side to side (fig. 14). When not in locomotion an amoeba often alternately draws in and thrusts out a pseudopodium on either side, advancing the one while withdrawing the other. This may take place at any point and may be repeated indefinitely. The floating forms have 3–6 short pseudopodia (fig. 13). In the flowing forms the cytoplasm is clearly differentiated into ectosarc and endosarc, the latter being conspicuously thick at the anterior end (figs. 12, 14). In floating forms no such differentiation was observed.

Binary fission.—During division the amoeba does not necessarily round up, although all movement of the organism ceases (fig. 16). The division of the nucleus is promitotic, that is, large chromatic polar masses are formed within the nuclear membrane and fibrils appear between them. The beginning of nuclear division is characterized by an increase in nuclear size (fig. 17). The nuclear membrane becomes more distinct and the peripheral chromatin more evident than in the resting nucleus. The central karyosome then

increases in size, elongates, and assumes a bent dumb-bell shape (fig. 18). The peripheral chromatin at the same time migrates in the nuclear membrane toward the karyosome. Spindle fibers then appear between the chromatic polar masses, which then move apart (figs. 19–22). The nuclear membrane constricts in the middle and the two daughter nuclei separate. The polar mass then assumes the shape of the karyosome, and the resting nucleus is reconstructed. Although I have searched very carefully for the chromosomes during the process of division, I have not been able to differentiate them from the spindle fibers. At the same time that reconstruction and separation are taking place in the nucleus, division of the cytoplasm starts. This is a simple constriction into approximately equal parts. Several instances of double nuclear division were found (fig. 23), and ordinarily the spindles lay parallel. In no case were more than two spindles found.

FORMATION OF GAMETES.—In old cultures the amoebae form gametes (fig. 69). The first stage in gamete formation is enlargement of the karyosome (fig. 28), which then fragments, the chromatin particles being extruded into the cytoplasm (figs. 29–32). These become collected into aggregates of chromatin surrounded by a clear area (fig. 30). The cytoplasm then contracts around these, so that a mass of small gametes is formed, each with an anterior nucleus (fig. 32). These gametes at first are very small and spherical in shape, with a poorly differentiated nucleus, which lies near the periphery. At this point the flagellum appears apparently growing out from the nucleus (fig. 33). I have concluded that the flagellum is located at the anterior end of the organism, since, whenever the organism moves the flagellum precedes it. Development of the flagellum is accompanied by increase in size of the gametes.

The gametes from many amoebae are released at the same time and form a swarm (fig. 69). In this stage they undergo a process which I believe to be conjugation. Two flagellates apparently conjugate to form a uniflagellate zygote. The stages of this process as found in prepared slides are shown in figs. 33, 69. I believe that the process is one of true conjugation rather than division, because it differs so radically from the stages of undoubted division which are described later. Although there is no conclusive evidence, I believe

that the zygote becomes uniflagellate by the fusion of the two gametic flagella. The change from the flagellate form to amoeba, or vice versa, can be induced by the addition of distilled water or Knop's solution to the culture, or by lowering its temperature.

FLAGELLATE STAGE

MORPHOLOGY.—The flagellates which arise from the amoebae are pyriform in shape and appear smaller than the amoebae. The younger flagellates differ somewhat from the older in size and shape, being larger on the average, and having a width of one-third the length of the body as compared with two-thirds in the older organisms. No differentiation into ectoplasm and endoplasm was observed.

The nucleus of the flagellate is like that of the amoeba, with a well defined membrane and central karyosome, and divides promitotically. The position of the nucleus in the cytoplasm is variable, generally being near the center of the organism, but some forms were found in which it had migrated into the anterior end. In all such cases the organisms were feeding very rapidly, and the flagella had become longer.

The single flagellum is about twice the length of the organism, and is situated at the extreme anterior end. It ends just inside the periphery in a deeply staining granule, the blepharoplast, which is connected with the karyosome by means of a rhizoplast.

Individuals were found with the nuclear membrane pulled out in the direction of the blepharoplast (fig. 37); also with a long flagellum and a nucleus lying close to the blepharoplast (fig. 57*a*). This increase in flagellar length is coincident with movement of the nucleus to a position near the blepharoplast. Judging from these observations, the blepharoplast may either be derived from the centriole or budded off from the karyosomic material around it. It arises from the karyosome and is always connected with it. After extension from the nucleus it takes up its position at the periphery of the cytoplasm coincident with the growth of the flagellum.

Under certain environmental conditions the flagellum is pulled back into the organism. This has been observed in living material, and the indications are that the material is returned to the nucleus. Reflagellation begins with a change of form (fig. 37).

The flagellate feeds in the same manner as the amoeba. Bacteria are always taken in at the posterior region, where they are digested in vacuoles (figs. 8–11).

BINARY FISSION.—The first step in binary fission is generally the division of the blepharoplast, although sometimes the development of the new flagellum precedes it. Next the nucleus divides mitotically, and last of all the cytoplasm divides longitudinally. Cytoplasmic division begins before the nuclear division at the anterior end and rapidly proceeds posteriorly. Late stages of division give the appearance of an elongate organism with an anterior and a posterior flagellum, but this is only a transitory state (figs. 40–46). When the division is complete the posterior ends round off and the daughter individuals move away from each other. In all cases the newer flagellum is less active than the older and becomes attached to débris, while the older moves with a characteristic jerky movement.

From observations made during the course of the investigation, it is apparent that the flagellates occur more commonly in the cultures than the amoebae. In a fresh culture made from spore cysts amoebae usually appear, but as the culture grows older the flagellates become more and more numerous.

Most of the amoebae in a culture can be transformed into flagellates in two hours by lowering the temperature. A little over an hour after a preparation has been made from a cooled culture, contracting amoebae with developing flagella are found in different places on the cover glass. These will be found to be more or less free from the substratum, and to be moving at the anterior end by a characteristic jerky movement, through a rather wide range of space. After the amoebae have been transformed into flagellates they send out and retract short pseudopodia almost continuously, and twist and turn, getting nearer all the time to the rounded up condition. When they reach a thick, long, pyriform shape they swim away with the narrow anterior end foremost (figs. 37–39).

In changing from the flagellate to the amoeboid stage, the organisms settle to the bottom, develop pseudopodia, retract their flagella, and assume the typical amoeboid form (fig. 47). This change can be induced by lowering the temperature or by mechanical disturbance. After such stimulation the flagellate begins to whirl

around until it seems exhausted, then settles to the bottom and assumes the amoeboid form.

Encystment

Encystment follows a period of rapid division, and appears to depend upon the heaviness of inoculation or upon the relative number of amoebae in the culture. The onset of the process is characterized by a cessation of division, as only an occasional division figure is found in preparations from cultures in which most of the individuals are encysting (fig. 70).

Organisms undergoing encystment are characterized by the entire absence of food vacuoles, by a rounding up of the cytoplasm, and by enlargement of the contractile vacuole with an increase in the rate of its pulsation (fig. 26). The protoplasm becomes dense, the contractile vacuole disappears, and a thin wall is formed around the organism. Later a second wall is formed within this, which is much thicker and contains no visible opening (fig. 34).

In some encystments a sporoblast is found filled with minute inclusions which may be "parasites" (fig. 36). Under proper conditions, such as the addition of fresh culture medium, these inclusions move about violently within the sporoblast, and finally rupture the cyst wall and are liberated (fig. 36). These inclusions appear to be similar to the dots seen in the plasmodia, nuclei, and chloroplasts of invaded tobacco tissues.

An amoeba that is emerging from the cyst may easily be recognized by the appearance of contractile vacuoles with a rapid rate of pulsation. The cyst wall disappears and a long pseudopodium is protruded. Following this the amoebae come together to form the plasmodium (figs. 60–64). This stage was observed in two cultures during January and April in 1924.

Plasmodium

The plasmodium appeared after the addition of fresh Knop's solution to cultures following encystment of the amoebae induced by evaporation. The amoebae which fuse to form the plasmodium are characterized by the presence of long stringy pseudopodia (figs. 60–64), relatively larger nuclei than those of the ordinary amoebae (fig. 62), the entire absence of food and contractile vacuoles (figs. 63,

64), and less differentiation between the ectoplasm and endoplasm (fig. 62). When pseudopodia of two of these organisms come in contact they fuse and the two amoebae flow together. By this process hundreds of amoebae run together to form a huge plasmodium (figs. 60–64). The plasmodium moves like a huge amoeba but more actively. The line of flow is generally in one direction, but the plasmodium may often change its course (figs. 65, 66).

The ectosarc is very clear and liquid, of unequal thickness over different parts of the organism, and generally abundant at the advancing margin. The nucleus of each amoeba that enters into the formation of the plasmodium retains its individuality, and no nuclear division has ever been observed, although there is a change just before the free spores are formed. In other words, each nucleus undergoes a reorganization before spore formation. The plasmodium takes in food by the flowing of the protoplasm around the object, but just prior to and during spore formation no food is ingested.

Immediately before the free spores are formed, the cytoplasm becomes clearer and more granular. The free spores are liberated in a row as the plasmodium moves along (fig. 67). Each free spore contains a single nucleus and a highly vacuolated cytoplasm (fig. 50). The nuclei of these spores enlarge, and chromidia are given off which form aggregates, around each of which a cyst wall is formed. Each free spore will produce three to fifteen of such sporocysts (figs. 54, 55).

Fig. 2 combines what I believe to be the sequence of forms in culture. Although I have obtained direct evidence for each sequence figured, further work may necessitate a number of minor changes. In this connection it should be noted that certain flagellates and amoebae of the types depicted in figs. 57–58*g* were observed in my cultures which cannot at present be fitted into any scheme of the life cycle. In considering the forms shown in fig. 2, it should be emphasized again that the only form which I have ever encountered in sections of the plant is the plasmodium (figs. 1, 3, 4, 59).

Possible relation to tobacco mosaic

The occurrence of the plasmodium of this organism in the cells of tobacco plants affected with mosaic-like symptoms raises the ques-

tion whether the organism or its inclusions or both are causally related to mosaic of tobacco. Only approximately twenty-five healthy and twenty-five diseased plants have been sectioned and studied so far. In none of the healthy plants was the organism found, nor was it obtained from them in culture; it was found in all of the diseased plants and also was obtained from them in culture.

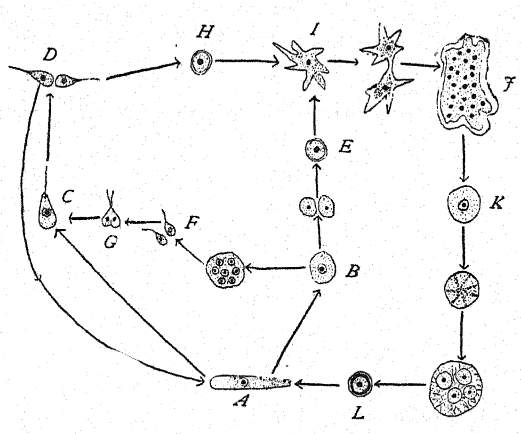

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FIG. 2.—Diagram of life cycle of *Plasmodiophora tabaci:* amoeboid stage (*A*) gives rise to other amoebae (*B*) by division, or is transformed directly into flagellate stage (*C*); amoebae (*B*) either encyst (*E*) or give rise to gametes (*F*) which fuse to form zygote (*G*); this gives rise to the flagellate (*C*) which gives rise to other flagellates by binary fission (*D*); these can either return to the amoeboid condition (*A*) or encyst (*H*); cysts (*E*, *H*) give rise to amoebae (*I*) which fuse to form plasmodium (*J*); plasmodium gives off free spores (*K*) which give rise to spore cysts (*L*); from these the amoebae (*A*) arise.

The fact that the plasmodium has not been found in plants free from mosaic-like symptoms is significant, although it does not prove that the organism is the pathogene.

The work done so far in culturing the organism and inoculating plants with these cultures is of a preliminary nature, and has not been critical enough to establish the causal relationship. Inoculations of healthy plants with cultures of the organism have produced the disease; the organism has been demonstrated in the tissue of the inoculated plants, and subsequently has been obtained from them in culture. The difficulty is, however, that these were not *single* pure

cultures, and that the possibility of contamination with a virus has not been eliminated. Two sets of experiments were conducted with nine plants each. In the first set, three plants were inoculated subepidermally with an agar-agar culture of bacteria obtained when affected leaves were placed in Knop's solution; three plants were inoculated with the culture of the flagellate stage of the organism, plus the bacteria which developed in culture from the plant tissue; and three plants were held as controls. Only the three plants inoculated with the mixed culture of flagellates and bacteria developed the disease. In the second experiment three plants were inoculated subepidermally with a mixed culture of bacteria and plasmodia; three were inoculated with a mixed culture of bacteria and flagellates; and three were held as controls. The plants inoculated with the plasmodium and those inoculated with the flagellates developed the disease twenty-one days after inoculation, while the controls remained healthy.

So far it has not been possible to grow the organism in cultures free of bacteria. If cultures are entirely free the organism does not live long. The bacteria seem to be a part of its food, and their relative abundance determines to a great extent the state in which the organism occurs in culture. Thus when bacteria become scarce in culture the flagellates and amoebae encyst; when they become abundant again the organisms excyst.

Work is in progress on the problems arising out of the findings reported in this paper.

Summary

1. A new species of a mycetozoan has been cultured from tobacco plants with mosaic-like symptoms.

2. Sections of leaves of tobacco plants affected with mosaic-like symptoms show the plasmodium stage of the organism within the plant cells. The peripheries of nuclei and of chloroplasts often show little dots which suggest the inclusions found in the cysts of the organism in culture.

3. In culture the mycetozoan has a flagellate and an amoeboid stage, and forms gametes, plasmodia, free spores, and cyst spores.

4. Certain inclusions are found in the cyst which may be "parasites" of the mycetozoan.

5. The mycetozoan described is provisionally placed in the genus *Plasmodiophora* and is named **P. tabaci.**

6. Amoebae and flagellates which differ somewhat from those of *P. tabaci* have been cultured in Knop's solution from tomato plants affected with mosaic, from potato plants affected with leaf roll, and from intestines of aphids feeding on diseased tobacco, tomato, and potato plants.

I wish to express my indebtedness to Professor G. N. CALKINS for many helpful criticisms and suggestions while this work was in progress at Columbia University. I am indebted to M. H. RUCKES for the photographs; to Miss D. DOWNIE for preparation of the drawings in figs. 1, 3, and 4; to Mrs. C. C. SPEIDEL for copying the line drawings; and to H. R. HALSEY.

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EXPLANATION OF PLATES XXXIV–XXXVII

PLATE XXXIV

FIG. 3.—Detail of plasmodium represented diagrammatically in fig. 1, drawn with camera lucida; ×about 1000.

FIG. 4.—Portion of huge plasmodium found in tissues of tobacco leaf: section cuts a vein obliquely and exposes a few spiral thickenings; plasmodium lies in phloem and adjoining tissues; some cell walls disappeared; engulfed in plasmodium are three host nuclei as well as chloroplasts in all stages of degeneracy; nuclei of plasmodium stand out clearly; drawn with camera lucida; ×about 1000.

FIGS. 5–58*g*.—All drawings made with camera lucida; ×1200.

FIGS. 5–11.—Organisms taken from one oil immersion field, showing change from flagellate to amoeba stage and method of feeding; figs. 5–7 are amoebae, and figs. 8–11 are flagellates taking in food; figs. 8, 10, flagellates dividing.

FIG. 12.—Amoebae in flowing (limax) stage.

FIG. 13.—Amoeba in floating form showing several small pseudopodia.

FIG. 14.—Amoeba showing pseudopodia produced by alternating flow.

FIG. 15.—Amoebae showing resting nucleus with central karyosome, clear space, and nuclear membrane with small amount of peripheral chromatin.

FIG. 16.—Amoeba rounding up preparatory to division.

FIG. 17.—Prophase showing elongated nucleus, vacuolated karyosome, and peripheral chromatin somewhat more evident than in resting stage.

FIG. 18.—Dumb-bell-shaped karyosome showing development of spindle.

FIG. 19.—Late prophase showing polar caps, granular chromatic polar masses, spindle fibers, and centrodesmus.

FIG. 20.—Early prophase showing constriction of nuclear membrane.

FIG. 21.—Anaphase showing chromatin masses moving apart.

FIG. 22.—Late anaphase showing condensation of chromatin to form daughter karyosomes.

FIG. 23.—Division of binucleate amoeba showing two parallel spindles.

FIG. 24.—Binucleate individual showing nuclear reconstruction.

FIG. 25.—Individual undergoing rapid division, showing division of daughter nuclei before constriction of cytoplasm is completed.

FIG. 26.—Encystment of amoeba, showing enlarged contractile vacuole characteristic of this stage.

Fig. 27.—Amoeba completely encysted.

FIG. 28.—Enlargement of karyosome preceding fragmentation.

PLATE XXXV

FIGS. 29–31.—Formation of gamete nuclei by fusion of extruded chromidia.

FIG. 32.—Later stages in gamete formation, showing breaking down of cell nucleus and formation of gamete nuclei.

FIG. 33.—Outgrowth of flagellum from gamete and subsequent fusion of gametes to form zygote.

FIG. 34.—Encysted amoeba showing thin and thick cyst wall.

FIG. 35.—Encysted amoeba showing thin cyst wall, and nucleus with large amount of peripheral chromatin.

FIG. 36.—Cysts with mature sporoblast containing inclusions (parasites?); cyst nucleus pressed flat against cyst wall.

FIG. 37.—Amoeba changing into flagellate stage, showing outgrowth of blepharoplast from karyosome of nucleus.

FIG. 38.—Flagellate stage showing completed flagellum connected with karyosome by means of rhizoplast.

FIG. 39.—Flagellate showing completed flagellum ending in blepharoplast.

FIG. 40.—Beginning of division of flagellate, showing division of blepharoplast while remainder of organism is still in resting stage.

FIG. 41.—Completed division of blepharoplast and flagellum, showing one daughter flagellum in active motion, other trailing it.

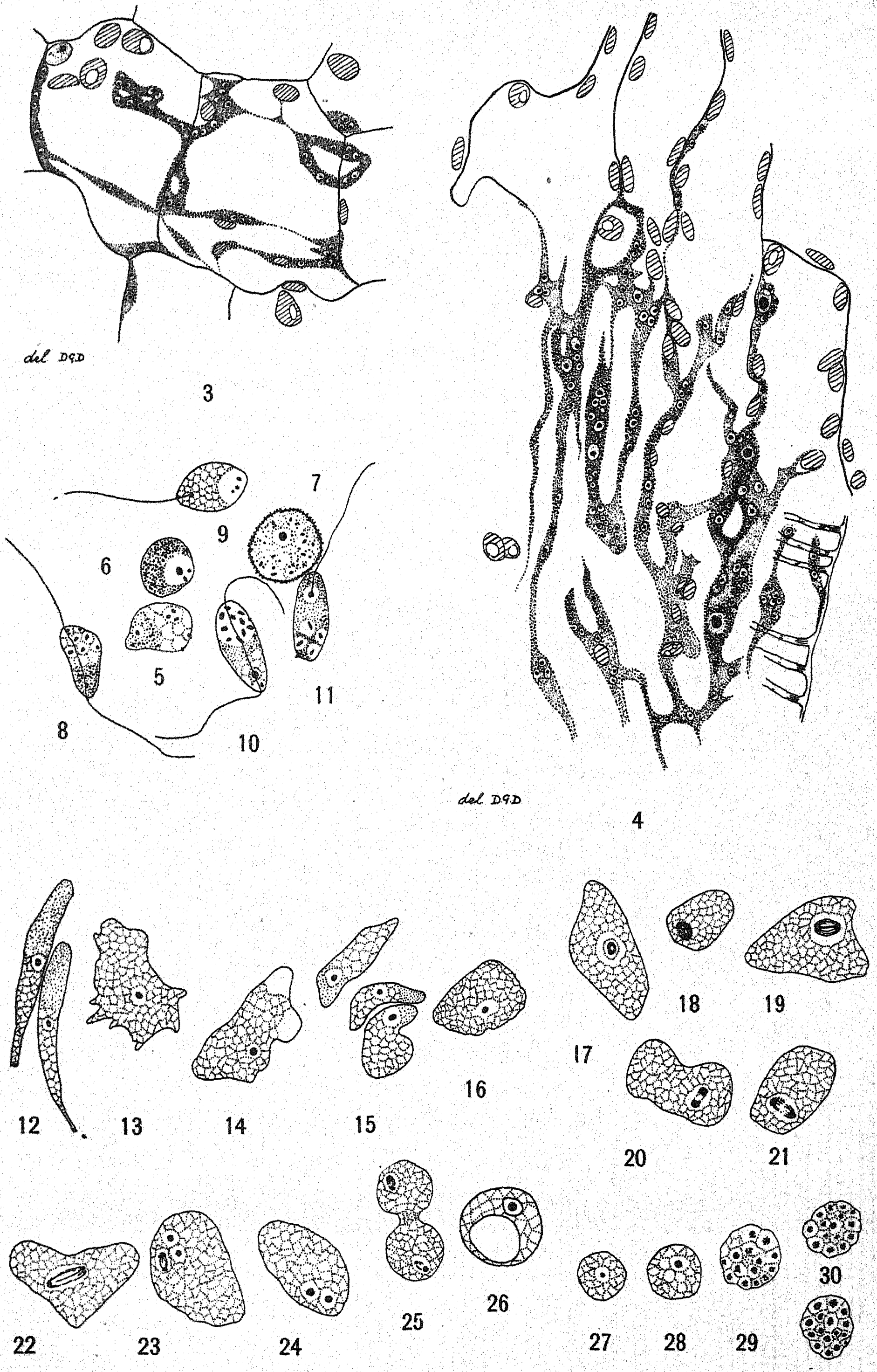
FIG. 42.—Beginning of cytoplasmic division; one of the two daughter blepharoplasts has a flagellum, other has not yet developed one.

FIG. 43.—Later stage in cytoplasmic division, with anterior ends of the two individuals pulled apart, giving appearance of an elongate individual with flagellum at each end.

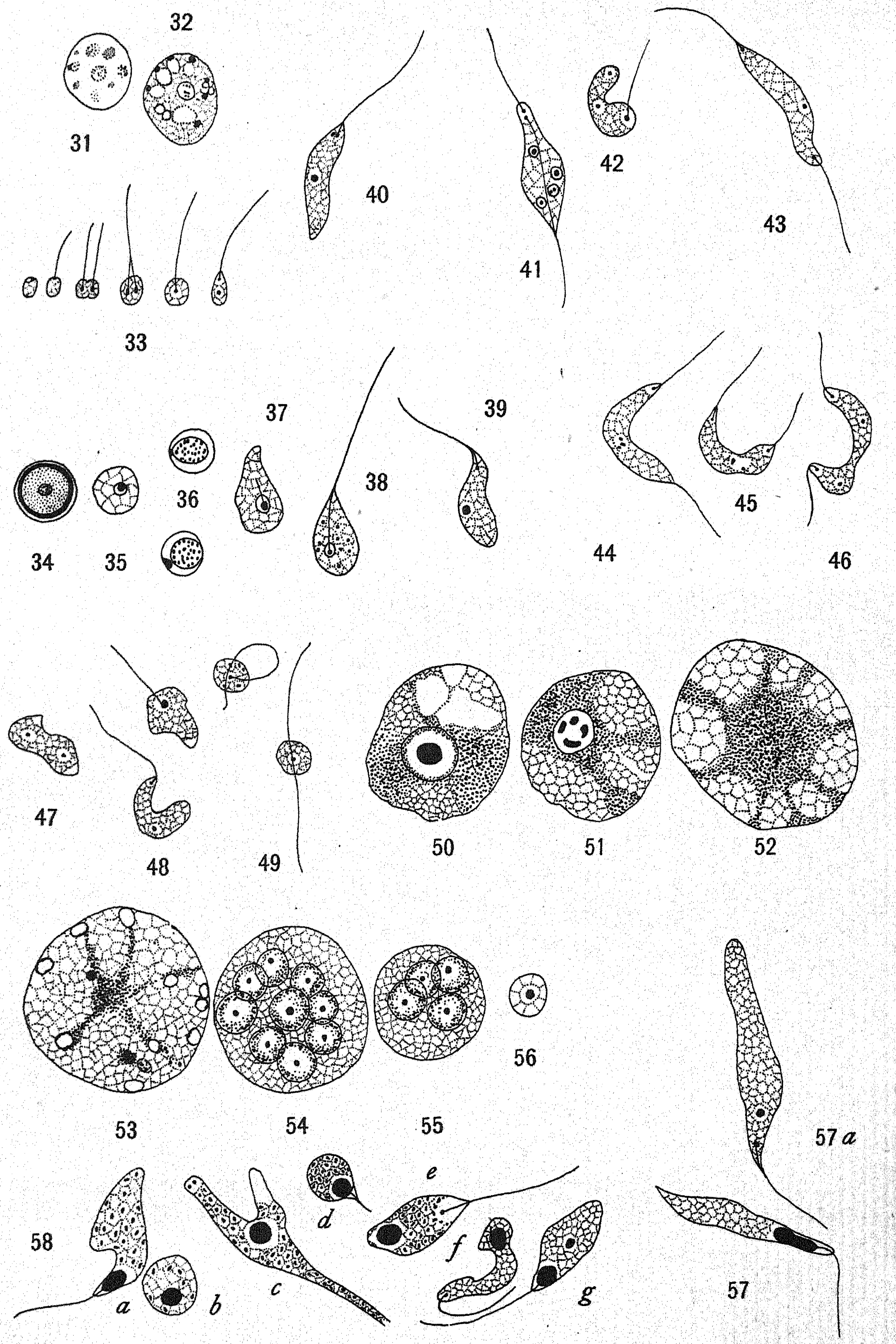
FIGS. 44, 45.—Two flagellates with anterior ends of daughter individuals separated, showing beginning of nuclear division.

FIG. 46.—Later stage in division, showing completed nuclear division and cytoplasm constricting.

FIG. 47.—Organism that began division in flagellate stage and is completing it in amoeboid stage; nuclear division completed and cytoplasmic division begun.



JONES on A MYCETOZOAN



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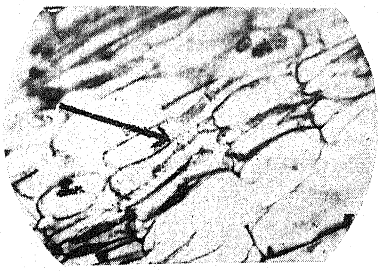

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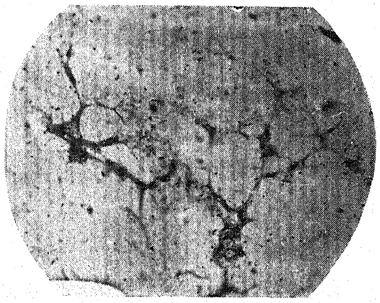

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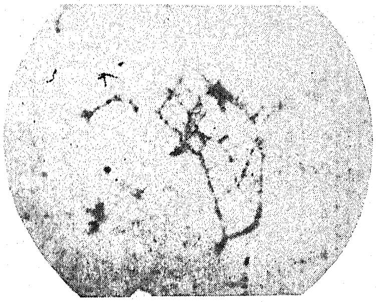

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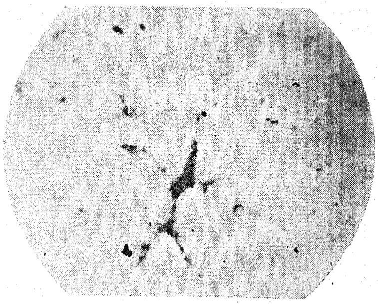

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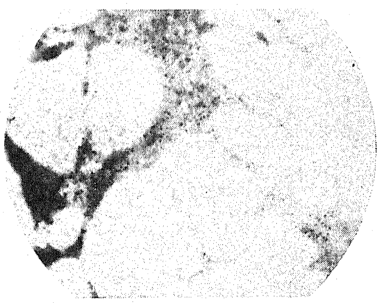

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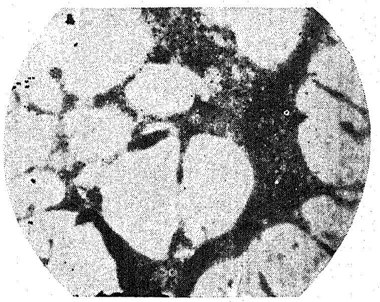

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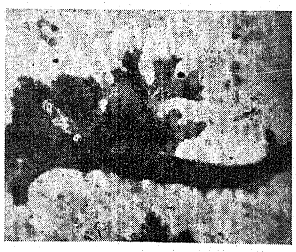

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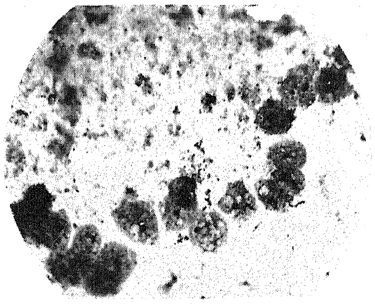

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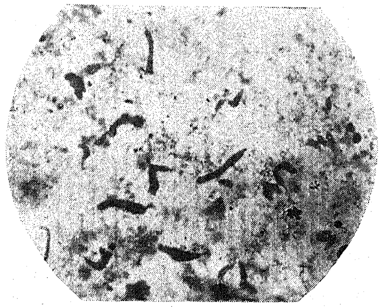

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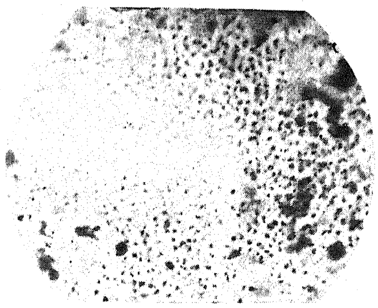

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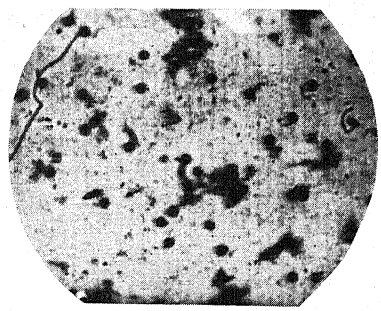

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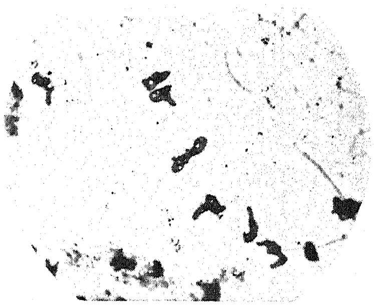

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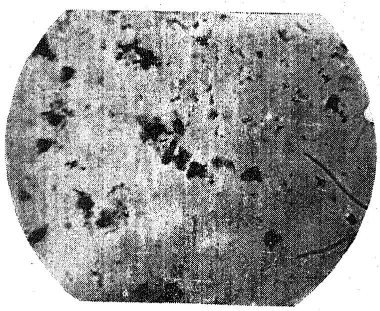

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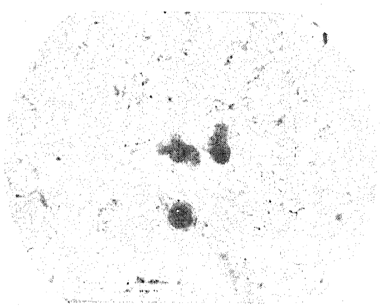

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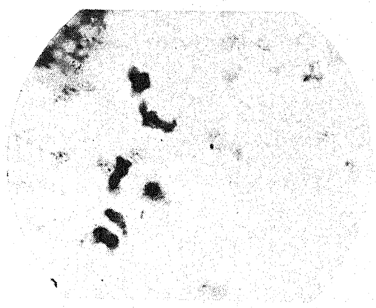

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JONES on A MYCETOZOAN

FIG. 48.—Flagellates changing into amoebae, showing flattening out of organism, taking in of flagellum, and outgrowth of pseudopodium.

FIG. 49.—Encystment of flagellate, showing withdrawal of flagellum and rounding up of organism.

FIG. 50.—Free spore deposited by plasmodium, showing beginning of chromodial extrusion.

FIGS. 51, 52.—Breaking down of spore nucleus and extrusion of chromidia.

FIGS. 53–55.—Formation of sporocysts within free spore.

FIG. 56.—Mature sporocyst.

FIG. 57.—Nucleus at anterior end in act of forming blepharoplast.

FIG. 57*a*.—Nucleus moving posteriorly after forming blepharoplast.

FIG. 58*a*.—Amoeba changing into flagellate; note large size of nucleus.

FIG. 58*b*.—Amoeba encysting with same type of enlarged nucleus.

FIG. 58*c*.—Flowing amoeba.

FIG. 58*d–g*.—Development of flagellum.

FIG. 58*e*, *f*.—Nucleus in posterior end (condition causing this change not known).

PLATE XXXVI

FIGS. 59–75.—Photomicrographs; ×about 350.

FIG. 59.—Section of root of tobacco plant, showing plasmodium in cortex.

FIGS. 60–64.—Fusion of amoebae to form plasmodia.

FIGS. 65–66.—Plasmodia.

PLATE XXXVII

FIG. 67.—Free spores deposited in a row by plasmodium.

FIG. 68.—Amoebae.

FIG. 69.—Gametes swarming and fusing.

FIG. 70.—Flagellates encysting and cysts.

FIG. 71.—Amoebae from tomato plants with mosaic symptoms.

FIG. 72.—Amoebae cultured from intestines of an aphid feeding on tomato plant with mosaic symptoms.

FIG. 74.—Amoebae cultured from potato plant with leaf roll symptoms.

FIG. 75.—Amoebae cultured from intestines of an aphid feeding on potato plant with leaf roll symptoms; figs. 60–75 from material grown in Knop's solution.

FLORA OF AN ILLINOIS COAL BALL

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 349

FREDDA DORIS REED

(WITH PLATE XXXVIII AND ONE FIGURE)

Introduction

The Carboniferous flora of Europe and England is quite well known, as the result of numerous investigations conducted for the most part within the last fifty years. Until recently (3), however, material for the study of the Carboniferous flora of America was not found in great quantities, except in the form of impressions. During the last year Dr. A. C. NOÉ, of the University of Chicago, has investigated coal fields of Illinois, Iowa, and Kentucky, and has collected an immense amount of material in the form of petrifactions, or coal balls as they are called technically, all of which contain sectionable plant tissue in varying quantities and in varying degrees of preservation. With this abundance of material, a Carboniferous flora of America as rich and diversified as that of Europe should be reconstructed. It seems advisable, therefore, to publish accounts of the various plant types as they are uncovered.

The plants described in this paper were found in one coal ball which came from the Calhoun Coal Company, Calhoun, Richland County, Illinois. The coal seam is no. 15, which belongs to the McLeansboro formation or the Upper Pennsylvanian of Illinois.

The ball measured approximately 5 cm. in diameter, and an analysis showed that it was composed largely of calcium carbonate. From the ball eighteen sections were made, all of which show plant tissue, but in many cases the tissues were too fragmentary and isolated to be of any value. Of the plant structures that could be identified, however, there was a remarkable variety ranging through the Pteridophytes and lower Gymnosperms.

Of the stems found only the stele was described, for the cortex was either not preserved at all or was too poorly preserved to permit description. Furthermore, only transverse sections were made, but

in most cases they were cut in a slightly oblique manner or were so thick that the markings on the walls of the tracheids were discernible.

Calamites

In a number of sections there is a portion of wood possessing an extensive secondary growth which has been identified as belonging in the *Calamites* group. This bit of wood appears to be one-fourth of a transverse section of a stele whose diameter would measure about 1.5 cm. As in most *Calamites* stems, there is very little pith present, and in this case there is no tissue preserved outside the secondary wood. The primary wood and the wood rays are quite well preserved, and show the anatomical features of certain *Calamites* species.

One of the most prominent characteristics of *Calamites* is the carinal canal found at the apex of each of the xylem wedges, and formed by the disorganization of the protoxylem; such a canal is quite distinctly seen in this specimen. In some of the bundles the canal borders directly on the pith; in other instances there is evidence of a row of small cells surrounding it, which are probably the remaining cells of the protoxylem, but in either case the development of the metaxylem must have been entirely centrifugal. Abutting on the metaxylem is the secondary wood, whose radial thickness consists of some eighty elements.

Extending between the xylem wedges are the parenchyma rays, composed of exceedingly wide and thin walled cells. These tapering rays are gradually shut off by the interfascicular wood.

The formation of a carinal canal, the absence of centripetal primary xylem, and the character of the principal wood rays are all peculiar to the *Calamites communis* (Binney) type (5), which appears so frequently in the English Coal Measures and is also the common type found in Europe. Since this American type possesses these same anatomical features the same name has been applied to it.

Sphenophyllum

Sphenophyllum has been found in the Devonian, appears throughout the Coal Measures, and extends into the Permian. Considering such a wide geological range, the anatomy of the stem is

remarkably uniform, for only two species based upon stem structure have been described: *Sphenophyllum insigne* and *S. plurifoliatum*, the former a Pre-carboniferous species and the latter common throughout the Coal Measures. The specimen found in this American coal ball, in so far as it is possible to describe it from a transverse section, agrees in every particular with that of *S. plurifoliatum*. SCOTT (6) has associated this form with that of the contemporary *S. myriophyllum*, known only from impressions.

In many of the sections there appear portions of the stele more or less complete, occasionally with bits of the cortex attached. Fig. 3 shows a section in which the stele is almost entirely preserved. Here it measures about 3 mm. in diameter. The photograph shows the solid strand of primary wood of triarch structure which was centripetally developed, for at the apex of each angle may be seen cells of much smaller caliber, which are the elements of the protoxylem. The organization of the secondary wood is of that type peculiar to this species: radiating out from the angles of the primary xylem are the tracheids of the fascicular wood, and between these groups are ones of larger tracheids, regularly and radially arranged, which are known as interfascicular wood. The tracheids of the primary and of the secondary wood are marked with multiseriate pits on the radial as well as on the tangential walls.

Between the truncated angles of the tracheids of the secondary wood are little groups of thin walled cells. From longitudinal sections these cells have been found to be vertical strands of parenchyma forming the medullary rays (7). The presence of these thin walled cells, making the medullary rays, and the absence of the carinal canals formed as in *Calamites* by the disorganization of the protoxylem, are the characteristics that distinguish this from the *Sphenophyllum insigne* type.

Bothrodendron

As long ago as 1833, LINDLEY and HUTTON (4) identified some impressions as *Bothrodendron*, but it was not until 1890 that the internal structure was known. At that time LOMAX found a stem that had the external appearance of *Bothrodendron*, and possessed the anatomical features of *Lepidodendron mundum* previously described

by WILLIAMSON (12), and so established *Bothrodendron mundum*. WILLIAMSON also described a species of *Stigmaria* which is apparently the underground or creeping portion of the stem.

In 1908 WATSON (9) gave a detailed account of a cone which, from the anatomy of the axis, he was able to relate to this species, and also determined that it was identical with a Lepidodendraceous cone figured and described by WILLIAMSON in 1880 (11). Furthermore, detached sporangia bearing spores described by WILLIAMSON (10) in 1878 belonged to this same species. Thus almost the entire plant has been described, and it is found to be one of the most ancient of Lycopods, for impressions of it have been found in the Devonian.

In this coal ball there were portions of the vegetative structure, a detached microsporangium and a megasporangium, both containing spores, and a number of isolated spores. The vegetative structure is a transverse section of a stem, and is illustrated in fig. 4. Judging from the size it must have been a stem tip, for the entire stem, including the cortex, measures only 2 mm. in diameter, while the diameter of the stele is approximately 0.8 mm. The stele is of the protostele type, but shows evidence of transition to the siphonostele condition, which is the prevailing type in the larger stems of *Bothrodendron*. The primary wood is composed of very large elements, exhibiting reticulated and pitted thickenings. External to the primary wood are the small cells of the protoxylem. There is no secondary growth.

The inner cortex has almost entirely disappeared, but the outer cortex is fairly well preserved. These cells are somewhat heavier walled than the few remaining cells of the inner cortex, and are irregularly placed.

The microsporangium shows the attachment to the sporophyll. One section (fig. 5) is almost a median cut of the sporophyll, for it shows the tracheids with spiral and scalariform thickenings; it is not quite median, however, for only a portion of the ligule appears. Near the base of the ligule, or rather near the ligular pit, may be seen some tracheids directed from the normal course of the bundle toward the ligule. This is not necessarily an indication that the ligule was supplied with a vascular strand, but that here is a situa-

tion analogous to that in *Selaginella*, in some species of which cells beneath the glossopodium become thickened, and in the mature condition are transformed into short tracheids, so that the leaf trace appears to be enlarged beneath the ligule (2).

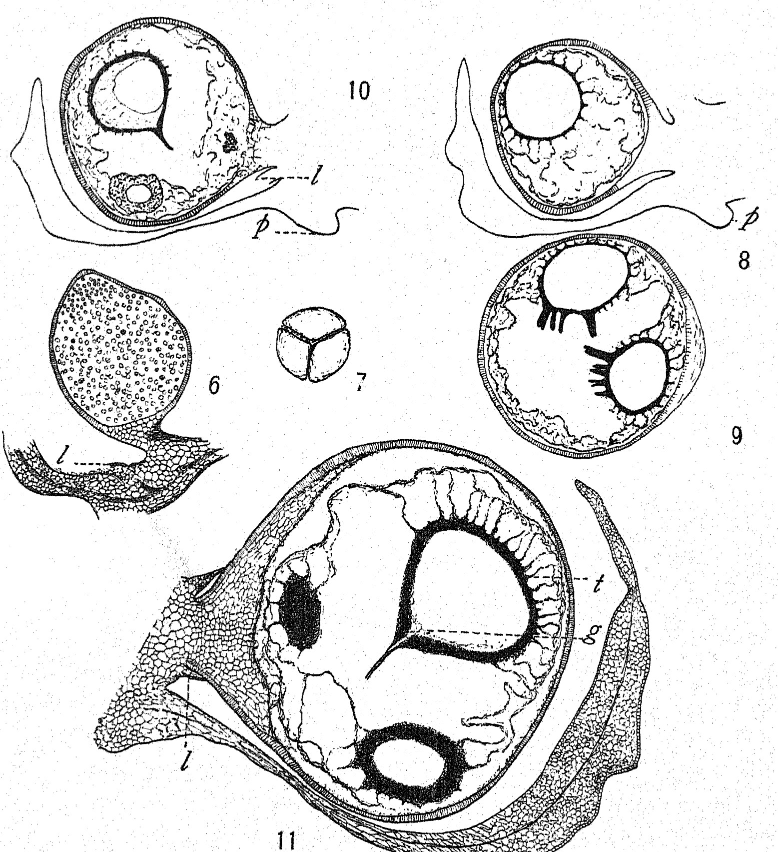
The sporangium is roughly spherical in form, but somewhat higher than it is wide, and measures about 2.5 mm. in diameter. The wall consists of one row of radially elongated cells. Inclosed in the sporangium are a great number of microspores in the tetrad stage, as illustrated in figs. 6 and 7. The diameter of the tetrads of spores varies from 0.05 to 0.06 mm. A rough calculation of the number of spores gives 80,000, which is a conservative figure. The microspores show no markings or sculpturing on the walls.

The section of the megasporangium is much more satisfactory than that of the microsporangium, in that it is much more complete. The material from which figs. 8–10 were made is not now available, for these are camera lucida drawings made during the preparation of the sections, and the material is now ground away.

The sporophyll does not show the attachment to the cone, but was apparently broken off quite near it. Fig. 10 shows the lobe on the lower part of the sporophyll, and a portion of the ligule (*l*), both of which features are described by WATSON (9). The course of the vascular strand is indicated by the large scalariform tracheids, which are found to extend about four-fifths the length of the lamina. This, however, does not mean that the vascular strand terminated here, but merely that the section is so cut that the exact termination does not show.

The megasporangium is more nearly spherical in form than the microsporangium, and is larger, being about 3 mm. in diameter. The pedicel of the sporangium is attached to the horizontal portion of the sporophyll, it is quite narrow and very short, and expands into the sporangium, its epidermal cells becoming the wall cells of the sporangium. The wall of the sporangium is composed of one layer of radially elongated cells as in the microsporangium. In some places, however, there are indications of other wall cells, which would imply that there may have been other layers of wall cells, and that most of them have been resorbed by the functioning megaspores.

Four megaspores were observed, three of which appear in fig. 11. The fourth one disappeared in the preparation of the section. One



FIGS. 6–11.—Fig. 6, microsporangium showing portion of sporophyll, course of vascular strand, ligule, and tetrads of spores; ×10. Fig. 7, tetrad of microspores; ×230. Fig. 8, diagram of megasporangium showing sporophyll with lobe or projection of lower side, and one of the four megaspores; ×9. Fig. 9, diagram of megasporangium showing two megaspores; ×9. Fig. 10, diagram of megasporangium: *l*, ligule; *p*, lobe on sporophyll; ×9. Fig. 11, megasporangium with three of the four megaspores: *v*, vascular strand; *l*, ligule; *t*, tapetal plasmodium; *g*, female gametophyte; somewhat diagrammatic; ×16.

of the most prominent features of the megaspores is the thick outer spore coat, reminding one of the spiny exospore of living species of *Selaginella*. The method of the formation of this spore coat is sug-

gested in fig. 11; the spores are surrounded by a protoplasmic mass which apparently is homologous with the tapetal plasmodium of living Pteridophytes, as *Salvinia* and *Selaginella;* from this mass strands of protoplasm extend to the periphery of the sporangium, indicating that the outer spore coat is laid down by the tapetal plasmodium.

Some of the megaspores contain some tissue, probably that of the female gametophyte. If one assumes that the development of the megaspore parallels that of the megaspores of *Selaginella*, then the female gametophyte at this stage would be either in the uninucleate stage or in the subsequent free nuclear condition, which condition seems to be confirmed by the fragile appearance of the contents of the megaspore in the megasporangium, as well as in many of the isolated megaspores, one of which is shown in fig. 13.

A comparison of the fruiting and vegetative structures of *Bothrodendron* and *Selaginella* suggests a very close relationship between the two groups. *Bothrodendron* is like *Selaginella* in the possession of a creeping stem, a ligule, and the development of the spores. The widest divergence is probably in the anatomy of the stem, for there is nothing in this ancient Lycopod that approaches the organization of the lacunar spaces found in *Selaginella*. The imperfect preservation of the inner cortex of *Bothrodendron*, however, in contrast with the outer cortex, is a condition which is quite regularly found in Paleozoic Lycopods, and may partly be due to imperfectly and irregularly formed intercellular spaces. BOWER (1) reports lacunar spaces with very well developed trabeculae in the axis of *Lepidostrobus Brownii*, concerning which he writes, "It will be seen that these trabeculae, originating as they do from the irregular sheath surrounding the bundle, are very similar to the trabeculae of *Selaginella*." *Selaginella*, therefore, is not peculiar in the possession of these features, but rather possesses well developed and highly specialized forms of lacunae and trabeculae, the prototypes of which may be found in the primitive Lycopods.

Lyginopteris

In addition to the preceding, there is yet another woody stem whose identity is more uncertain than those previously described,

although the evidence seems to place it in the lower Gymnosperms. The stem or stele is illustrated in fig. 14. As may be seen, it is not round but slightly flattened in outline, its larger diameter measuring 4 mm. In the center there is a group of relatively thin walled cells, rather sharply delimited from the cells of the primary xylem, making the medulla or pith. This central strand or pith is surrounded by the primary xylem, which is organized in six or seven bundles. The tracheids of the primary xylem are marked with multiseriate pits on all walls alike, and they inclose smaller cells with spiral and scalariform thickenings of the protoxylem. These smaller tracheids are situated nearer the periphery of the primary xylem than the pith, but in any event the bundle is mesarch. The development of the primary wood was so extensive that it shut out the principal rays, and formed a continuous zone of wood around the pith.

Bordering on the primary is the secondary wood, whose radial thickness varies from five elements on the shorter radius to eight on the longer radius. Like the tracheids of the primary wood, these have multiseriate pits, but unlike the primary wood, the pits are confined to the radial faces.

Outside the zone of secondary wood the only tissue remaining is that of the leaf traces. The section is so thick and the preservation is so poor that it is impossible to orient the elements of these traces, but the gross topography shows that in general those traces which border the secondary wood are united, while those farther away from the stele have separated and are double. None of the leaf traces shows any secondary wood.

The double leaf traces, the type and disposition of the thickenings of the secondary wood, and the mesarch character of the protoxylem are all gymnospermic features, and may be found in the Lyginopterideae (**8**), so that one could not be far wrong in applying the generic name *Lyginopteris*. As for a specific name, it is hoped that further investigation will bring to light sufficient additional information to attach one.

Discussion

The reconstruction of any particular fossil plant must necessarily be a gradual process, since it is only rarely that the stem and roots or stem and leaves are found attached; or again, it is only in excep-

tional cases that impressions can be related to internal structure. For example, both *Calamites* stems and roots were known for some time before a specimen was found which made it possible to relate the two, and so the stem was given the name *Calamites*, but the root was described under the term *Astromyelon;* likewise, the leaves were known as *Annularia*. Even now that *Calamites*, *Astromyelon*, and *Annularia* are known to belong to one and the same plant, the terms persist more or less. Such a process, while obviously quite necessary, has resulted in a multiplicity of terms which are somewhat confusing. It was partly because of this situation that the use of new terms was avoided in this paper.

Again, the information concerning these plants, while sufficient for placing them in certain definite groups, is really quite meager; nothing is known of the external appearance, of the underground portions, of the leaves, and, except in the case of *Bothrodendron*, of the fructifications. Until these features are known and the plants can be definitely established, it seems best to resort to the nomenclature already in use.

Summary

In the coal ball described were portions of plants that have hitherto been known from the Carboniferous of America only as impressions; they are *Calamites*, *Sphenophyllum*, *Bothrodendron*, and *Lyginopteris*. Only transverse sections of the steles of *Calamites*, *Sphenophyllum*, and *Lyginopteris* were found. Belonging to *Bothrodendron* are transverse sections of a stem tip, a megasporangium and microsporangium both attached to sporophylls and containing spores, and isolated megaspores. In the microsporangium the spores are in the tetrad stage, while in the megasporangium four megaspores were observed. The most striking feature in the comparison of *Bothrodendron* and *Selaginella* is the similarity of the two, which indeed compels the conclusion that *Bothrodendron* is the progenitor of the living species of *Selaginella*.

Acknowledgment is due to Dr. A. C. Noé, who furnished the material and under whose direction the investigation was made.

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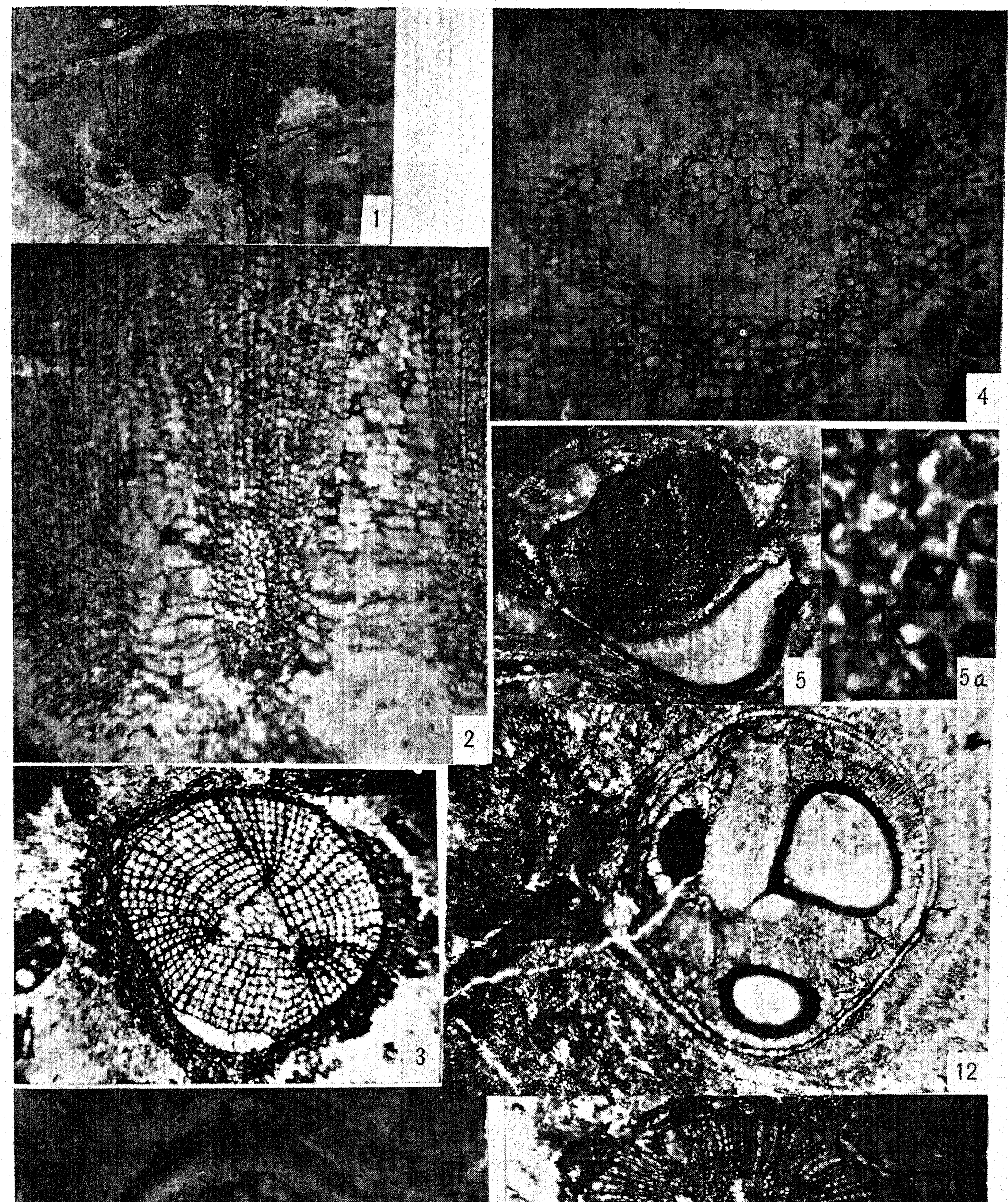

REED on COAL BALL

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4. LINDLEY, J., and HUTTON, W., The fossil flora of Great Britain. London. *pls. 80, 81.* 1833.
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10. WILLIAMSON, W. C., On the organization of the fossil plants of the Coal Measures. Phil. Trans. Roy. Soc. London. **169**:319. 1878.
11. ———, Organization of the fossil plants of the Coal Measures. Phil. Trans. Roy. Soc. London. **171**:493. 1880.
12. ———, Organization of the fossil plants of the Coal Measures. Phil. Trans. Roy. Soc. London. **180**:155. 1889.

EXPLANATION OF PLATE XXXVIII

FIG. 1.—Portion of transverse section of stele of *Calamites;* ×10.

FIG. 2.—Detail of one of bundles from fig. 1, showing few pith cells, carinal canal, extensive development of secondary wood, and primary wood rays; ×16.

FIG. 3.—Transverse section of stele of *Sphenophyllum;* ×12.

FIG. 4.—Transverse section of stem tip of *Bothrodendron: oc*, outer cortex; *ic*, inner cortex; *px*, protoxylem; ×28.

FIG. 5.—Section of microsporangium containing tetrads of microspores; ×12.

FIG. 5*a*.—Photomicrograph of tetrads of spores shown in fig. 5; ×150.

FIG. 12.—Photomicrograph of megasporangium containing three megaspores; ×16.

FIG. 13.—One of isolated megaspores containing some tissue; ×32.

FIG. 14.—Transverse section of stele of *Lygínopteris;* ×12.

CURRENT LITERATURE

BOOK REVIEWS

Plant pathology

Before reviewing some new books in this field, reference should be made to new editions of well established ones, for these have to some extent passed the test of time. The leading one is SORAUER'S[1] *Handbuch*, the most comprehensive of plant pathology texts. It is suggestive that the first two volumes are in the fifth edition and the other three in the fourth. The book is a dynamic factor which no pathologist can ignore. The merit of the first volume is its comprehensive point of view and its great fund of information; its weakness lies in its diffuseness. The latter probably is inherent in the subject matter, and is due in part to the fact that we are so little acquainted with the normal structure, physiology, and ecology of each of the numerous species of plants. The task of the plant pathologist is far more difficult than that of the human pathologist, because the field of the latter is much more restricted. There is an increasing use of American literature in the new editions, and consequently the book is becoming more useful by giving more truly the present status of phytopathological knowledge.

KÜSTER'S[2] *Pathologische Pflanzenanatomie* in its third edition is a complete revision, and maintains its leadership in the field of pathological plant anatomy. The presentation is so thorough and exhaustive that it is of use, not only to those interested in abnormal anatomy, but also to workers in the field of normal plant anatomy. The book consists of a special and a general part. In the former are considered (1) marbling, (2) etiolation, (3) hyperhydric tissues, (4) wound tissues and regeneration, and (5) galls. In the general part are taken up (1) histogenesis, (2) developmental mechanics, and (3) ecology of pathological tissues. KÜSTER'S book has exerted a very considerable influence upon American plant pathologists, whose contributions in turn are increasingly finding their way into his admirable book.

Plant disease fungi, by STEVENS,[3] as its title indicates, deals mostly with fungi which cause plant diseases and very little with diseases. It is essentially

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[1] SORAUER, P., Handbuch der Pflanzenkrankheiten. Vol. I, 5th ed. 1925; Vol. II, 5th ed. 1925; Vol. III, 4th ed. 1923; Vol. IV, 4th ed. 1925; Vol. V, 4th ed. 1925. Berlin: Paul Parey.

[2] KÜSTER, ERNST, Pathologische Pflanzenanatomie in ihren Grundzügen. 3rd ed. pp. xii+558. *figs. 285*. Jena: Gustav Fischer. 1925.

[3] STEVENS, F. L., Plant disease fungi. pp. 469. *figs. 407*. New York: Macmillan Co. 1925.

a condensation of his previous book, restricting its scope to the more important pathogenic fungi which occur in the United States. The keys have not been made as workable, however, as one might hope for. It is true, of course, as STEVENS stated in his earlier book, that the "present unsatisfactory condition of taxonomy of the fungi, loose and imperfect description of species, disregarded generic limitations" make it difficult to produce workable keys. For these reasons it is all the more important that an author should strive for a certain amount of consistency of terminology in compiling a book of this nature. On page 116, in the key to the Phacidiaceae, the character "stroma" is used to separate the genera *Keithia* and *Coccomyces* from *Rhytisma*, the former lacking a stroma. On page 119 the legend of figure 115 is as follows: "An ascocarp of *C. hiemalis* on its thick stroma." Slips of this type detract from the usefulness of the book.

It is regrettable that the nomenclature of the bacteria was disturbed. The juggling of names of the Schizomycetes is of doubtful value at present, especially for beginners. It is especially regrettable that each shift must carry with it the name of the juggler. Of course, the outcome in the long run may be fortunate, because indulgence in this procedure will not stop until the situation becomes an unbearable nuisance. It is unfortunate that the older literature used in compiling the book is not cited as in the earlier volume. The book is printed on good paper and the figures show considerable detail.

In their laboratory outlines, WHETZEL, HESLER, GREGORY, and RANKIN[4] depart from the traditional American point of view of phytopathology, by attempting a classification of plant diseases on a pathological rather than a mycological or host basis. This is a welcome departure, and is a hopeful sign that possibly in the future, courses in plant pathology will deal with the subject matter of phytopathology, that is, plant responses. In the past fungi have been the central theme and plant responses merely interesting or necessary secondary considerations. There are difficulties in any system of classification. The system adopted in this manual is no exception. The difficulty in using necrotic, hypoplastic, and hyperplastic as categories in classifying diseases lies in the fact that the cellular responses may be of one type, whereas the response of the plant as an organismal unit, which after all is the essential entity from the theoretical and practical point of view, may be quite different. Thus in club root of cabbage the cellular response is hyperplastic, whereas the response of the plant as a whole is hypoplastic and necrotic.

The preface of the book contains a sentence in which we have the teaching philosophy of WHETZEL. He states: "Although the acquisition of a body of facts is an important and necessary part of the work in such a course, a more vital feature is the training in logical methods of acquiring them." The manual lives up to this high purpose to a remarkable extent.

[4] WHETZEL, H. H., HESLER, L. R., GREGORY, C. T., and RANKIN, W. H., Laboratory outlines in plant pathology. pp. 231. Philadelphia and London: W. B. Saunders Co. 1925.

WHETZEL's manual is in part the result of a pedagogical experiment which he has been carrying on for some time. Simplification of the data of a science for pedagogical reasons always carries with it potentialities of danger. The phenomena of plant diseases, like many others in biology, cannot always be put into hard and fast logical compartments. The manual bristles with new words and phrases. In coining hard and fast definitions care should be used. WHETZEL takes a forward step in differentiating between the concepts "parasite" and "pathogene," but it is questionable whether the terms "pathogenesis" and "saprogenesis" have been wisely chosen. If pathogene means an "organism capable of causing disease," pathogenesis should mean the process of causing disease and saprogenesis the process of causing decay. These terms are not necessarily mutually exclusive, although they are so used in the manual. In the manual, according to the glossary, pathogenesis is taken to be that portion or phase of the life-cycle of a pathogene when it is associated with a living "suscept," whereas saprogenesis is taken to be the portion or phase of the life-cycle of a pathogene when it is not in association with the "suscept." These definitions in turn are based upon the coining of a new term suscept, and the dropping of the current botanical usage of the term life-cycle and adopting in its place that used by some workers in the fields of bacteriology and public health.

The book contains a wealth of information which is exceedingly useful to the teacher. For the beginner it possibly contains too much information. The student often is told in great detail to *observe* things which are minutely described in the manual, so that there is danger that he will develop habits of depending upon the manual and neglecting study of the material itself.

Imprisonment in a camp in Egypt during the war gave MORSTATT[5] an opportunity to produce an extremely worthwhile book. It lends itself well to use as a textbook for introduction into the theoretical and practical aspects of plant pathology. The book is only 159 pages long and necessarily sketchy. Chapter I, Die Erkennung der Pflanzenkrankheiten, takes up (1) the symptoms, and (2) methods of investigation and descriptions of diseases. Chapter II, Krankheitslehre, is extremely interesting and stimulating. It takes up (1) the concepts and nature of plant diseases, (2) pathological plant anatomy, and (3) pathological plant physiology. Chapter III, Die Ursachen der Pfanzenkrankheiten, presents in Part I (1) the general aspects of such topics as parasitism, symbiosis, specialization and transmission; followed by a synoptic discussion of (A) pathogenic plants, and (B) pathogenic and injurious animals. Filterable viruses as causative agents of plant diseases are treated in an appendix to this chapter. In the second part of Chapter III, there is a sketch of the non-living factors which cause diseases. Chapter IV, Pflanzenschutz, gives a brief summary of control measures. The book is a very successful attempt to present plant pathology as a unified field of applied botany.

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[5] MORSTATT, H., Einführung in die Pflanzenpathologie. V. pp. 159. *figs. 4.* Berlin: Gebrüder Borntraeger. 1923.

OWENS,[6] in his laboratory outlines, uses suggestive questions to stimulate the student. The outlines, however, suffer from brevity. OWENS very clearly conceives plant pathology as an applied field of botany, and consequently about three-fourths of his text is devoted to discussion of plant disease control. Under this topic he takes up the biological principles (such as parasitism and immunity) which have bearing upon control. Although there is recognition of the fact that plant pathology is more than a branch of mycology, the book is permeated by a mycological atmosphere. For each disease presented there is given a series of quotations from the relevant literature, saving much time for the student. It gives him some experience in reading the literature first-hand, and yet spares him the task of working through the vast amount of irrelevant and repetitious material found in many publications on plant diseases.

CHUPP,[7] has written a much needed book on the diseases of truck and garden crops. Emphasis is given to discussion of symptoms and control measures. The causal aspects, however, are not ignored. The book fits well into the Rural Manual Series, and will be extremely useful to growers and to extension workers. Students in plant pathology will find it a handy reference book. An enormous amount of work has been necessary to compile the diffused and widely scattered literature bearing upon diseases of vegetables.

CUNNINGHAM'S[8] *Practical bacteriology* is intended primarily for students of agriculture. It is a small book but is well written. It will prove useful primarily to bacteriologists. Chapter V (five pages) is devoted to the bacteriology of plant diseases.—G. K. K. LINK.

Biology of lichens

An interesting book on the biology of lichens has been written by TOBLER.[9] The material is presented under the following headings: (1) development and growth, (2) physiology, (3) ecology, and (4) symbiosis. TOBLER champions the concept "lichens." He believes that it is a useful concept and that its retention will stimulate morphological, physiological, and taxonomic studies of the component algae and fungi. TOBLER contends that a "lichen" is a new organism which has arisen out of the symbiotic relationship of an alga and a fungus, this organism typically being characterized by a new and distinct form, the lichen thallus, and even more significantly by a new and typical metabolism, the formation of lichen acids. Furthermore, this typical metabolism occurs only in

[6] OWENS, C. E., Principles of plant pathology. A text and laboratory manual (mimeographed). Part I, pp. 126. Part II, pp. 288. Edwards Brothers, Ann Arbor, Michigan. 1924.

[7] CHUPP, C., Manual of vegetable-garden diseases. pp. xxiv+647. *figs. 155.* New York: Macmillan Co. 1925.

[8] CUNNINGHAM, A., Practical bacteriology. pp. vi+188. *figs. 14.* Edinburgh: Oliver and Boyd. 1924.

[9] TOBLER, F., Biologie der Flechten. pp. vii+265. one colored plate. *figs. 67.* Berlin: Gebrüder Borntraeger. 1925.

those lichens in which the symbiotic relationship has led to the typical morphogenic result. He contends that lichens are a group in active evolution, many members not yet having reached that level of symbiosis which is characterized by new and distinct form and metabolism. He takes decided but good tempered issue with FINK's suggestion that the concept "lichens" be dropped and the lichens be placed with the fungi.—G. K. K. LINK.

NOTES FOR STUDENTS

Filamentous algae of Iowa.—TIFFANY[10] has published a very full account of the filamentous algae collected in northwestern Iowa, illustrated by 16 plates. The collections were made briefly during late spring, summer, and early autumn, so that the whole growing season was represented. The total number of forms recorded is 200, the Myxophyceae including 35 and the Chlorophyceae 165. The Oedogoniaceae are particularly well represented, and are presented in more detail than the other groups, including 75 forms, with keys and plates in addition to the descriptions. The region of collection was in the "northwestern corner" of the state, where a summer laboratory is maintained by the University of Iowa. Within a short distance of the laboratory there are numerous lakes, swamps, streams, and drainage ditches, presenting a diversity of habitats rich in fresh water algae.—J. M. C.

Mosaic diseases.—FERNOW[11] has published some important results of an investigation of mosaic diseases. He conducted inoculation experiments on 19 host species, 15 of which were Solanaceae. All but one of these species proved to be susceptible to one or more of the 8 mosaics found to be distinct. These mosaics were distinguished by the species attacked, and by the different symptoms when inoculated on the same species. When a host is attacked by two or more of these mosaics, failure to distinguish them doubtless accounts for many of the discrepancies in the literature of the subject. This segregation of mosaics into distinct species is an important contribution to the investigation of this perplexing group.—J. M. C.

[10] TIFFANY, L. H., The filamentous algae of northwestern Iowa with special reference to the Oedogoniaceae. Trans. Amer. Micr. Soc. **45**:69–132. 1926.

[11] FERNOW, K. H., Interspecific transmission of mosaic diseases of plants. Memoir 96, Cornell Univ. Agric. Exper. Sta. December 1925.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold-face** type; synonyms in *italics*.